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(54) **PLANTS HAVING INCREASED YIELD-RELATED TRAITS AND A METHOD FOR MAKING THE SAME**

(57) The present invention relates generally to the field of molecular biology and concerns a method for increasing various plant yield-related traits by modulating expression in a plant of a nucleic acid sequence encoding a yield increasing polypeptide selected from the group consisting of:

an AT-hook motif nuclear localized 19/20 (AHL19/20), a GRP (Growth Regulating Protein) (wherein said GRP polypeptide is a metallothionein 2a (MT2a) polypeptide), an alanine aminotransferase (AAT)-like polypeptide, and an alanine aminotransferase (AAT) polypeptide.

The present invention also concerns plants having

modulating expression of a nucleic acid sequence encoding a yield increasing polypeptide selected from the group consisting of: an AT-hook motif nuclear localized 19/20 (AHL19/20), a GRP (Growth Regulating Protein) (wherein said GRP polypeptide is a metallothionein 2a (MT2a) polypeptide), an alanine aminotransferase (AAT)-like polypeptide, and an alanine aminotransferase (AAT) polypeptide which plants have increased yield-related traits relative to control plants. The invention also provides constructs useful in the methods of the invention.

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Description

[0001] The present invention relates generally to the field of molecular biology and concerns a method for increasing various plant yield-related traits by increasing expression in a plant of a nucleic acid sequence encoding a yield-increasing polypeptide selected from the group consisting of:

an AT-hook motif nuclear localized 19/20 (AHL19/20),
 a GRP (Growth Regulating Protein, wherein said GRP polypeptide is a metallothionein 2a (MT2a) polypeptide),
 an alanine aminotransferase (AAT)-like polypeptide, and
 an alanine aminotransferase (AAT) polypeptide. The present invention also concerns plants having increased expression of a nucleic acid sequence encoding said yield increasing polypeptide, which plants have increased yield-related traits relative to control plants. The invention also provides constructs useful in the methods of the invention.

[0002] The ever-increasing world population and the dwindling supply of arable land available for agriculture fuels research towards increasing the efficiency of agriculture. Conventional means for crop and horticultural improvements utilise selective breeding techniques to identify plants having desirable characteristics. However, such selective breeding techniques have several drawbacks, namely that these techniques are typically labour intensive and result in plants that often contain heterogeneous genetic components that may not always result in the desirable trait being passed on from parent plants. Advances in molecular biology have allowed mankind to modify the germplasm of animals and plants. Genetic engineering of plants entails the isolation and manipulation of genetic material (typically in the form of DNA or RNA) and the subsequent introduction of that genetic material into a plant. Such technology has the capacity to deliver crops or plants having various improved economic, agronomic or horticultural traits.

[0003] A trait of particular economic interest is increased yield. Yield is normally defined as the measurable produce of economic value from a crop. This may be defined in terms of quantity and/or quality. Yield is directly dependent on several factors, for example, the number and size of the organs, plant architecture (for example, the number of branches), seed production, leaf senescence and more. Root development, nutrient uptake, stress tolerance and early vigour may also be important factors in determining yield. Optimizing one or more of the abovementioned factors may therefore contribute to increasing crop yield.

[0004] Seed yield is a particularly important trait, since the seeds of many plants are important for human and animal nutrition. Crops such as corn, rice, wheat, canola and soybean account for over half the total human caloric intake, whether through direct consumption of the seeds themselves or through consumption of meat products raised on processed seeds. They are also a source of sugars, oils and many kinds of metabolites used in industrial processes. Seeds contain an embryo (the source of new shoots and roots) and an endosperm (the source of nutrients for embryo growth during germination and during early growth of seedlings). The development of a seed involves many genes, and requires the transfer of metabolites from the roots, leaves and stems into the growing seed. The endosperm, in particular, assimilates the metabolic precursors of carbohydrates, oils and proteins and synthesizes them into storage macromolecules to fill out the grain.

[0005] Harvest index, the ratio of seed yield to aboveground dry weight, is relatively stable under many environmental conditions and so a robust correlation between plant size and grain yield can often be obtained (e.g. Rebetzke et al 2002 Crop Science 42:739). These processes are intrinsically linked because the majority of grain biomass is dependent on current or stored photosynthetic productivity by the leaves and stem of the plant (Gardener et al 1985 Physiology of Crop Plants. Iowa State University Press, pp68-73). Therefore, selecting for plant size, even at early stages of development, has been used as an indicator for future potential yield (e.g. Tittone et al 2005 Agric Ecosys & Environ 105: 213). When testing for the impact of genetic differences on stress tolerance, the ability to standardize soil properties, temperature, water and nutrient availability and light intensity is an intrinsic advantage of greenhouse or plant growth chamber environments compared to the field. However, artificial limitations on yield due to poor pollination due to the absence of wind or insects, or insufficient space for mature root or canopy growth, can restrict the use of these controlled environments for testing yield differences. Therefore, measurements of plant size in early development, under standardized conditions in a growth chamber or greenhouse, are standard practices to provide indication of potential genetic yield advantages.

[0006] Another trait of importance is that of improved abiotic stress tolerance. Abiotic stress is a primary cause of crop loss worldwide, reducing average yields for most major crop plants by more than 50% (Wang et al. (2003) Planta 218: 1-14). Abiotic stresses may be caused by drought, salinity, extremes of temperature, chemical toxicity, excess or deficiency of nutrients (macroelements and/or microelements), radiation and oxidative stress. The ability to increase plant tolerance to abiotic stress would be of great economic advantage to farmers worldwide and would allow for the cultivation of crops during adverse conditions and in territories where cultivation of crops may not otherwise be possible.

[0007] Another important trait for many crops is early vigour. Improving early vigour is an important objective of modern rice breeding programs in both temperate and tropical rice cultivars. Long roots are important for proper soil anchorage in water-seeded rice. Where rice is sown directly into flooded fields, and where plants must emerge rapidly through

water, longer shoots are associated with vigour. Where drill-seeding is practiced, longer mesocotyls and coleoptiles are important for good seedling emergence. The ability to engineer early vigour into plants would be of great importance in agriculture. For example, poor early vigor has been a limitation to the introduction of maize (*Zea mays* L.) hybrids based on Corn Belt germplasm in the European Atlantic.

[0008] A further important trait is that of enhanced yield-related traits of plants grown under abiotic stress conditions. Abiotic stress is a primary cause of crop loss worldwide, reducing average yields for most major crop plants by more than 50% (Wang et al., *Planta* (2003) 218: 1-14). Abiotic stresses may be caused by drought, salinity, extremes of temperature, chemical toxicity, excess or lack of nutrients (macroelements and/or microelements), radiation and oxidative stress. The ability to enhance yield-related traits of plants grown under abiotic stress conditions would be of great economic advantage to farmers worldwide and would allow for the cultivation of crops during adverse conditions and in territories where cultivation of crops may not otherwise be possible.

[0009] Crop yield may therefore be increased by optimising one of the above-mentioned factors.

[0010] Depending on the end use, the modification of certain yield traits may be favoured over others. For example for applications such as forage or wood production, or bio-fuel resource, an increase in the vegetative parts of a plant may be desirable, and for applications such as flour, starch or oil production, an increase in seed parameters may be particularly desirable. Even amongst the seed parameters, some may be favoured over others, depending on the application. Various mechanisms may contribute to increasing seed yield, whether that is in the form of increased seed size or increased seed number.

[0011] One approach to increase yield-related traits (seed yield and/or biomass) in plants may be through modification of the inherent growth mechanisms of a plant, such as the cell cycle or various signalling pathways involved in plant growth or in defense mechanisms.

[0012] It has now been found that various seed yield-related traits may be increased in plants relative to control plants, without delayed flowering, by increasing expression in a plant of a nucleic acid sequence encoding an AT-hook motif nuclear localized 19/20 (AHL19/20) polypeptide. The increased seed-yield related traits comprise one or more of: increased number of flowers per panicle, increased total seed yield per plant, increased number of filled seeds, and increased harvest index.

[0013] It has further now been found that increasing expression of a nucleic acid sequence encoding a GRP polypeptide, wherein said GRP polypeptide is a metallothionein 2a (MT2a) polypeptide, gives plants grown under abiotic stress conditions having enhanced yield-related traits, relative to control plants grown under comparable conditions.

[0014] Additionally, it has now been found that modulating expression in above ground plant parts of a nucleic acid encoding an AAT-like polypeptide gives plants having enhanced yield-related traits, in particular increased yield relative to control plants.

[0015] Further it has now been found that yield-related traits in plants grown under non-nitrogen limiting conditions may be enhanced by modulating expression in such plants of a nucleic acid encoding an AAT polypeptide.

Background

[0016] DNA-binding proteins are proteins that comprise any of many DNA-binding domains and thus have a specific or general affinity to DNA. DNA-binding proteins include for example transcription factors that modulate the process of transcription, nucleases that cleave DNA molecules, and histones that are involved in DNA packaging in the cell nucleus.

[0017] The AT-hook motif is a short DNA binding protein motif that was first described in the high mobility group non-histone chromosomal proteins, HMG-I/Y (Reeves and Nissen (1990) *J Biol Chem* 265: 8573-8582). The AT-hook is known to interact with the minor groove of AT-rich nucleic acid sequences (Huth et al. (1997) *Nat Struc Biol* 4: 657-665). AT-hook motifs have been identified in a wide variety of DNA binding proteins from animals, plants and microorganisms. Unlike several well-characterized DNA binding motifs, the AT-hook motif is short, up to 13 amino acid residues, and has a typical tripeptide sequence with a glycine-arginine-proline (Gly-Arg-Pro or GRP) at its center.

[0018] In *Arabidopsis thaliana*, approximately 30 polypeptides, comprising at least one AT-hook motif, further comprise a plant and prokaryotes conserved (PPC) domain, which is described as DUF296 (domain of unknown function 296) in the InterPro domain database of the European Bioinformatics Institute (EBI) (Fujimoto et al. (2004) *Plant Molec Biol* 56: 225-239). One of these proteins was found to be localized in the nucleoplasm, and therefore named AT-hook motif nuclear localized protein 1 (AHL1; Fujimoto *et al.*, *supra*). The paralogous polypeptides were similarly named, i.e. AHL, and numbered consecutively.

[0019] In US patent 7,193,129, and in US patent application 2005/0097638, an *Arabidopsis thaliana* AHL polypeptide, AHL19 (according to Fujimoto *et al.*, *supra*) (identified as G2153) was transformed into *Arabidopsis*, and expressed using the 35S CaMV promoter. Transgenic plants showed modified traits, such as increased salt stress resistance, increased osmotic stress resistance, increased drought resistance, increased tolerance to freezing and increased plant response to sugars. In US patent application 2005/0097638, overexpression (under the control of a 35S CaMV promoter) of AHL19 polypeptide, as well as of several paralogous AHL polypeptides, significantly delayed flowering in the transgenic

plants compared to control plants, thereby increasing yield.

Summary

[0020] According one embodiment, there is provided a method for increasing seed yield-related traits in plants relative to control plants, comprising increasing expression of a nucleic acid sequence encoding an AHL19/20 polypeptide in a plant. The increased seed yield-related traits, comprise one or more of: increased number of flowers per panicle, increased total seed yield per plant, increased number of filled seeds, and increased harvest index.

[0021] According to one embodiment, there is provided a method for enhancing yield-related traits of a plant grown under abiotic stress conditions relative to control plants, comprising increasing expression of a nucleic acid sequence encoding a GRP polypeptide in a plant, wherein said GRP polypeptide is a metallothionein 2a (MT2a) polypeptide. The enhanced yield-related traits are one or more of: increased aboveground biomass, increased total seed yield per plant, increased number of filled seeds, increased total number of seeds, increased number primary panicles, and increased seed fill rate.

[0022] According one embodiment of the invention, there is provided a method for enhancing yield-related traits in plants relative to control plants, comprising modulating expression in above ground plant parts of a nucleic acid encoding an AAT-like polypeptide. In a preferred embodiment, expression of a nucleic acid encoding an AAT-like polypeptide is modulated (preferably increased) by operably linking the nucleic acid to a promoter active in above ground plant parts.

[0023] According one embodiment, there is provided a method for enhancing yield related traits in plants grown under non-nitrogen limiting conditions, comprising modulating expression of a nucleic acid encoding an AAT polypeptide in a plant.

Definitions

Polypeptide(s)/Protein(s)

[0024] The terms "polypeptide" and "protein" are used interchangeably herein and refer to amino acids in a polymeric form of any length, linked together by peptide bonds.

Polynucleotide(s)/Nucleic acid(s)/Nucleic acid sequence (s)/nucleotide sequence(s)

[0025] The terms "polynucleotide(s)", "nucleic acid sequence(s)", "nucleotide sequence(s)", "nucleic acid(s)" are used interchangeably herein and refer to nucleotides, either ribonucleotides or deoxyribonucleotides or a combination of both, in a polymeric unbranched form of any length.

Control plant(s)

[0026] The choice of suitable control plants is a routine part of an experimental setup and may include corresponding wild type plants or corresponding plants without the gene of interest. The control plant is typically of the same plant species or even of the same variety as the plant to be assessed. The control plant may also be a nullizygote of the plant to be assessed. A "control plant" as used herein refers not only to whole plants, but also to plant parts, including seeds and seed parts.

Homologue(s)

[0027] "Homologues" of a protein encompass peptides, oligopeptides, polypeptides, proteins and enzymes having amino acid substitutions, deletions and/or insertions relative to the unmodified protein in question and having similar biological and functional activity as the unmodified protein from which they are derived.

[0028] A deletion refers to removal of one or more amino acids from a protein.

[0029] An insertion refers to one or more amino acid residues being introduced into a predetermined site in a protein. Insertions may comprise N-terminal and/or C-terminal fusions as well as intra-sequence insertions of single or multiple amino acids. Generally, insertions within the amino acid sequence will be smaller than N- or C-terminal fusions, of the order of about 1 to 10 residues. Examples of N- or C-terminal fusion proteins or peptides include the binding domain or activation domain of a transcriptional activator as used in the yeast two-hybrid system, phage coat proteins, (histidine)-6-tag, glutathione S-transferase-tag, protein A, maltose-binding protein, dihydrofolate reductase, Tag•100 epitope, c-myc epitope, FLAG®-epitope, lacZ, CMP (calmodulin-binding peptide), HA epitope, protein C epitope and VSV epitope.

[0030] A substitution refers to replacement of amino acids of the protein with other amino acids having similar properties (such as similar hydrophobicity, hydrophilicity, antigenicity, propensity to form or break α -helical structures or β -sheet

structures). Amino acid substitutions are typically of single residues, but may be clustered depending upon functional constraints placed upon the polypeptide; insertions will usually be of the order of about 1 to 10 amino acid residues. The amino acid substitutions are preferably conservative amino acid substitutions. Conservative substitution tables are well known in the art (see for example Creighton (1984) *Proteins*. W.H. Freeman and Company (Eds) and Table 1 below).

Table 1: Examples of conserved amino acid substitutions

Residue	Conservative Substitutions	Residue	Conservative Substitutions
Ala	Ser	Leu	Ile; Val
Arg	Lys	Lys	Arg; Gln
Asn	Gln; His	Met	Leu; Ile
Asp	Glu	Phe	Met; Leu; Tyr
Gln	Asn	Ser	Thr; Gly
Cys	Ser	Thr	Ser; Val
Glu	Asp	Trp	Tyr
Gly	Pro	Tyr	Trp; Phe
His	Asn; Gln	Val	Ile; Leu
Ile	Leu, Val		

[0031] Amino acid substitutions, deletions and/or insertions may readily be made using peptide synthetic techniques well known in the art, such as solid phase peptide synthesis and the like, or by recombinant DNA manipulation. Methods for the manipulation of DNA sequences to produce substitution, insertion or deletion variants of a protein are well known in the art. For example, techniques for making substitution mutations at predetermined sites in DNA are well known to those skilled in the art and include M13 mutagenesis, T7-Gen in vitro mutagenesis (USB, Cleveland, OH), QuickChange Site Directed mutagenesis (Stratagene, San Diego, CA), PCR-mediated site-directed mutagenesis or other site-directed mutagenesis protocols.

Derivatives

[0032] "Derivatives" include peptides, oligopeptides, polypeptides which may, compared to the amino acid sequence of the naturally-occurring form of the protein, such as the protein of interest, comprise substitutions of amino acids with non-naturally occurring amino acid residues, or additions of non-naturally occurring amino acid residues. "Derivatives" of a protein also encompass peptides, oligopeptides, polypeptides which comprise naturally occurring altered (glycosylated, acylated, prenylated, phosphorylated, myristoylated, sulphated etc.) or non-naturally altered amino acid residues compared to the amino acid sequence of a naturally-occurring form of the polypeptide. A derivative may also comprise one or more non-amino acid substituents or additions compared to the amino acid sequence from which it is derived, for example a reporter molecule or other ligand, covalently or non-covalently bound to the amino acid sequence, such as a reporter molecule which is bound to facilitate its detection, and non-naturally occurring amino acid residues relative to the amino acid sequence of a naturally-occurring protein.

[0033] Furthermore, "derivatives" also include fusions of the naturally-occurring form of the protein with tagging peptides such as FLAG, HIS6 or thioredoxin (for a review of tagging peptides, see Terpe, *Appl. Microbiol. Biotechnol.* 60, 523-533, 2003).

Orthologue(s)/Paralogue(s)

[0034] Orthologues and paralogues encompass evolutionary concepts used to describe the ancestral relationships of genes. Paralogues are genes within the same species that have originated through duplication of an ancestral gene; orthologues are genes from different organisms that have originated through speciation, and are also derived from a common ancestral gene.

Domain

[0035] The term "domain" refers to a set of amino acids conserved at specific positions along an alignment of sequences of evolutionarily related proteins. While amino acids at other positions can vary between homologues, amino acids that are highly conserved at specific positions indicate amino acids that are likely essential in the structure, stability or function of a protein. Identified by their high degree of conservation in aligned sequences of a family of protein homologues, they can be used as identifiers to determine if any polypeptide in question belongs to a previously identified polypeptide family.

Motif/Consensus sequence/Signature

[0036] The term "motif" or "consensus sequence" or "signature" refers to a short conserved region in the sequence of evolutionarily related proteins. Motifs are frequently highly conserved parts of domains, but may also include only part of the domain, or be located outside of conserved domain (if all of the amino acids of the motif fall outside of a defined domain).

Hybridisation

[0037] The term "hybridisation" as defined herein is a process wherein substantially homologous complementary nucleotide sequences anneal to each other. The hybridisation process can occur entirely in solution, i.e. both complementary nucleic acid molecules are in solution. The hybridisation process can also occur with one of the complementary nucleic acid molecules immobilised to a matrix such as magnetic beads, Sepharose beads or any other resin. The hybridisation process can furthermore occur with one of the complementary nucleic acid molecules immobilised to a solid support such as a nitro-cellulose or nylon membrane or immobilised by e.g. photolithography to, for example, a siliceous glass support (the latter known as nucleic acid sequence arrays or microarrays or as nucleic acid sequence chips). In order to allow hybridisation to occur, the nucleic acid molecules are generally thermally or chemically denatured to melt a double strand into two single strands and/or to remove hairpins or other secondary structures from single stranded nucleic acid molecules.

[0038] The term "stringency" refers to the conditions under which a hybridisation takes place. The stringency of hybridisation is influenced by conditions such as temperature, salt concentration, ionic strength and hybridisation buffer composition. Generally, low stringency conditions are selected to be about 30°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. Medium stringency conditions are when the temperature is 20°C below T_m , and high stringency conditions are when the temperature is 10°C below T_m . High stringency hybridisation conditions are typically used for isolating hybridising sequences that have high sequence similarity to the target nucleic acid sequence. However, nucleic acid sequences may deviate in sequence and still encode a substantially identical polypeptide, due to the degeneracy of the genetic code. Therefore medium stringency hybridisation conditions may sometimes be needed to identify such nucleic acid sequence molecules.

[0039] The T_m is the temperature under defined ionic strength and pH, at which 50% of the target sequence hybridises to a perfectly matched probe. The T_m is dependent upon the solution conditions and the base composition and length of the probe. For example, longer sequences hybridise specifically at higher temperatures. The maximum rate of hybridisation is obtained from about 16°C up to 32°C below T_m . The presence of monovalent cations in the hybridisation solution reduce the electrostatic repulsion between the two nucleic acid sequence strands thereby promoting hybrid formation; this effect is visible for sodium concentrations of up to 0.4M (for higher concentrations, this effect may be ignored). Formamide reduces the melting temperature of DNA-DNA and DNA-RNA duplexes with 0.6 to 0.7°C for each percent formamide, and addition of 50% formamide allows hybridisation to be performed at 30 to 45°C, though the rate of hybridisation will be lowered. Base pair mismatches reduce the hybridisation rate and the thermal stability of the duplexes. On average and for large probes, the T_m decreases about 1°C per % base mismatch. The T_m may be calculated using the following equations, depending on the types of hybrids:

1) DNA-DNA hybrids (Meinkoth and Wahl, Anal. Biochem., 138: 267-284, 1984):

$$T_m = 81.5^\circ\text{C} + 16.6 \times \log_{10}[\text{Na}^+]^a + 0.41\%[\text{G/C}^b] - 500 \times [\text{L}^c]^{-1} - 0.61\% \text{ formamide}$$

2) DNA-RNA or RNA-RNA hybrids:

$$T_m = 79.8 + 18.5 (\log_{10}[\text{Na}^+]^a) + 0.58 (\% \text{G/C}^b) + 11.8 (\% \text{G/C}^b)^2 - 820/\text{L}^c$$

3) oligo-DNA or oligo-RNA^d hybrids:

For <20 nucleotides: $T_m = 2 (I_n)$

For 20-35 nucleotides: $T_m = 22 + 1.46 (I_n)$

^a or for other monovalent cation, but only accurate in the 0.01-0.4 M range.

^b only accurate for %GC in the 30% to 75% range.

^c L = length of duplex in base pairs.

^d oligo, oligonucleotide; I_n = effective length of primer = $2 \times (\text{no. of G/C}) + (\text{no. of A/T})$.

[0040] Non-specific binding may be controlled using any one of a number of known techniques such as, for example, blocking the membrane with protein containing solutions, additions of heterologous RNA, DNA, and SDS to the hybridisation buffer, and treatment with Rnase. For non-homologous probes, a series of hybridizations may be performed by varying one of (i) progressively lowering the annealing temperature (for example from 68°C to 42°C) or (ii) progressively lowering the formamide concentration (for example from 50% to 0%). The skilled artisan is aware of various parameters which may be altered during hybridisation and which will either maintain or change the stringency conditions.

[0041] Besides the hybridisation conditions, specificity of hybridisation typically also depends on the function of post-hybridisation washes. To remove background resulting from non-specific hybridisation, samples are washed with dilute salt solutions. Critical factors of such washes include the ionic strength and temperature of the final wash solution: the lower the salt concentration and the higher the wash temperature, the higher the stringency of the wash. Wash conditions are typically performed at or below hybridisation stringency. A positive hybridisation gives a signal that is at least twice of that of the background. Generally, suitable stringent conditions for nucleic acid sequence hybridisation assays or gene amplification detection procedures are as set forth above. More or less stringent conditions may also be selected. The skilled artisan is aware of various parameters which may be altered during washing and which will either maintain or change the stringency conditions.

[0042] For example, typical high stringency hybridisation conditions for DNA hybrids longer than 50 nucleotides encompass hybridisation at 65°C in 1x SSC or at 42°C in 1x SSC and 50% formamide, followed by washing at 65°C in 0.3x SSC. Examples of medium stringency hybridisation conditions for DNA hybrids longer than 50 nucleotides encompass hybridisation at 50°C in 4x SSC or at 40°C in 6x SSC and 50% formamide, followed by washing at 50°C in 2x SSC. The length of the hybrid is the anticipated length for the hybridising nucleic acid. When nucleic acid molecules of known sequence are hybridised, the hybrid length may be determined by aligning the sequences and identifying the conserved regions described herein. 1x SSC is 0.15M NaCl and 15mM sodium citrate; the hybridisation solution and wash solutions may additionally include 5x Denhardt's reagent, 0.5-1.0% SDS, 100 µg/ml denatured, fragmented salmon sperm DNA, 0.5% sodium pyrophosphate.

[0043] For the purposes of defining the level of stringency, reference can be made to Sambrook et al. (2001) Molecular Cloning: a laboratory manual, 3rd Edition, Cold Spring Harbor Laboratory Press, CSH, New York or to Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989 and yearly updates).

Splice variant

[0044] The term "splice variant" as used herein encompasses variants of a nucleic acid sequence in which selected introns and/or exons have been excised, replaced, displaced or added, or in which introns have been shortened or lengthened. Such variants will be ones in which the biological activity of the protein is substantially retained; this may be achieved by selectively retaining functional segments of the protein. Such splice variants may be found in nature or may be manmade. Methods for predicting and isolating such splice variants are well known in the art (see for example Foissac and Schiex (2005) BMC Bioinformatics 6: 25).

Allelic variant

[0045] Alleles or allelic variants are alternative forms of a given gene, located at the same chromosomal position. Allelic variants encompass Single Nucleotide Polymorphisms (SNPs), as well as Small Insertion/Deletion Polymorphisms (INDELs). The size of INDELs is usually less than 100 bp. SNPs and INDELs form the largest set of sequence variants in naturally occurring polymorphic strains of most organisms.

Gene shuffling/Directed evolution

[0046] Gene shuffling or directed evolution consists of iterations of DNA shuffling followed by appropriate screening and/or selection to generate variants of nucleic acid sequences or portions thereof encoding proteins having a modified biological activity (Castle et al., (2004) Science 304(5674): 1151-4; US patents 5,811,238 and 6,395,547).

Regulatory element/Control sequence/Promoter

[0047] The terms "regulatory element", "control sequence" and "promoter" are all used interchangeably herein and are to be taken in a broad context to refer to regulatory nucleic acid sequences capable of effecting expression of the sequences to which they are ligated. The term "promoter" typically refers to a nucleic acid sequence control sequence located upstream from the transcriptional start of a gene and which is involved in recognising and binding of RNA polymerase and other proteins, thereby directing transcription of an operably linked nucleic acid. Encompassed by the aforementioned terms are transcriptional regulatory sequences derived from a classical eukaryotic genomic gene (including the TATA box which is required for accurate transcription initiation, with or without a CCAAT box sequence) and additional regulatory elements (i.e. upstream activating sequences, increasers and silencers) which alter gene expression in response to developmental and/or external stimuli, or in a tissue-specific manner. Also included within the term is a transcriptional regulatory sequence of a classical prokaryotic gene, in which case it may include a -35 box sequence and/or -10 box transcriptional regulatory sequences. The term "regulatory element" also encompasses a synthetic fusion molecule or derivative that confers, activates or increases expression of a nucleic acid sequence molecule in a cell, tissue or organ.

[0048] A "plant promoter" comprises regulatory elements, which mediate the expression of a coding sequence segment in plant cells. The "plant promoter" preferably originates from a plant cell, e.g. from the plant which is transformed with the nucleic acid sequence to be expressed in the inventive process and described herein. This also applies to other "plant" regulatory signals, such as "plant" terminators. The promoters upstream of the nucleotide sequences useful in the methods of the present invention can be modified by one or more nucleotide substitution(s), insertion(s) and/or deletion(s) without interfering with the functionality or activity of either the promoters, the open reading frame (ORF) or the 3'-regulatory region such as terminators or other 3' regulatory regions which are located away from the ORF. It is furthermore possible that the activity of the promoters is increased by modification of their sequence, or that they are replaced completely by more active promoters, even promoters from heterologous organisms. For expression in plants, the nucleic acid sequence molecule must, as described above, be linked operably to or comprise a suitable promoter which expresses the gene at the right point in time and with the required spatial expression pattern.

[0049] For the identification of functionally equivalent promoters, the promoter strength and/or expression pattern of a candidate promoter may be analysed for example by operably linking the promoter to a reporter gene and assaying the expression level and pattern of the reporter gene in various tissues of the plant. Suitable well-known reporter genes include for example beta-glucuronidase or beta-galactosidase. The promoter activity is assayed by measuring the enzymatic activity of the beta-glucuronidase or beta-galactosidase. The promoter strength and/or expression pattern may then be compared to that of a reference promoter (such as the one used in the methods of the present invention). Alternatively, promoter strength may be assayed by quantifying mRNA levels or by comparing mRNA levels of the nucleic acid sequence used in the methods of the present invention, with mRNA levels of housekeeping genes such as 18S rRNA, using methods known in the art, such as Northern blotting with densitometric analysis of autoradiograms, quantitative real-time PCR or RT-PCR (Heid et al., 1996 Genome Methods 6: 986-994). Generally by "weak promoter" is intended a promoter that drives expression of a coding sequence at a low level. By "low level" is intended at levels of about 1/10,000 transcripts to about 1/100,000 transcripts, to about 1/500,000 transcripts per cell. Conversely, a "strong promoter" drives expression of a coding sequence at high level, or at about 1/10 transcripts to about 1/100 transcripts to about 1/1000 transcripts per cell. Generally, by "medium strength promoter" is intended a promoter that drives expression of a coding sequence at a level that is in all instances below that obtained under the control of a 35S CaMV promoter.

Operably linked

[0050] The term "operably linked" as used herein refers to a functional linkage between the promoter sequence and the gene of interest, such that the promoter sequence is able to initiate transcription of the gene of interest.

Constitutive promoter

[0051] A "constitutive promoter" refers to a promoter that is transcriptionally active during most, but not necessarily all, phases of growth and development and under most environmental conditions, in at least one cell, tissue or organ. Table 2a below gives examples of constitutive promoters.

Table 2a: Examples of plant constitutive promoters

Gene Source	Reference
Actin	McElroy et al, Plant Cell, 2: 163-171, 1990

(continued)

Gene Source	Reference
HMGB	WO 2004/070039
GOS2	de Pater et al, Plant J Nov;2(6):837-44, 1992, WO 2004/065596
Ubiquitin	Christensen et al, Plant Mol. Biol. 18: 675-689, 1992
Rice cyclophilin	Buchholz et al, Plant Mol Biol. 25(5): 837-43, 1994
Maize H3 histone	Lepetit et al, Mol. Gen. Genet. 231:276-285, 1992
Alfalfa H3 histone	Wu et al. Plant Mol. Biol. 11:641-649, 1988
Actin 2	An et al, Plant J. 10(1); 107-121, 1996
Rubisco small subunit	US 4,962,028
OCS	Leisner (1988) Proc Natl Acad Sci USA 85(5): 2553
SAD1	Jain et al., Crop Science, 39 (6), 1999: 1696
SAD2	Jain et al., Crop Science, 39 (6), 1999: 1696
V-ATPase	WO 01/14572
G-box proteins	WO 94/12015

Ubiquitous promoter

[0052] A ubiquitous promoter is active in substantially all tissues or cells of an organism.

Developmentally-regulated promoter

[0053] A developmentally-regulated promoter is active during certain developmental stages or in parts of the plant that undergo developmental changes.

Inducible promoter

[0054] An inducible promoter has induced or increased transcription initiation in response to a chemical (for a review see Gatz 1997, Annu. Rev. Plant Physiol. Plant Mol. Biol., 48:89-108), environmental or physical stimulus, or may be "stress-inducible", i.e. activated when a plant is exposed to various stress conditions, or a "pathogen-inducible" i.e. activated when a plant is exposed to exposure to various pathogens.

Organ-specific/Tissue-specific promoter

[0055] An organ-specific or tissue-specific promoter is one that is capable of preferentially initiating transcription in certain organs or tissues, such as the leaves, roots, seed tissue etc. For example, a "root-specific promoter" is a promoter that is transcriptionally active predominantly in plant roots, substantially to the exclusion of any other parts of a plant, whilst still allowing for any leaky expression in these other plant parts. Promoters able to initiate transcription in certain cells only are referred to herein as "cell-specific".

[0056] Examples of root-specific promoters are listed in Table 2b below:

Table 2b: Examples of root-specific promoters

Gene Source	Reference
RCc3	Plant Mol Biol. 1995 Jan;27(2):237-48

(continued)

Gene Source	Reference
Arabidopsis PHT1	Kovama et al., 2005; Mudge et al. (2002, Plant J. 31:341)
Medicago phosphate transporter	Xiao et al., 2006
Arabidopsis Pyk10	Nitz et al. (2001) Plant Sci 161(2): 337-346
root-expressible genes	Tingey et al., EMBO J. 6: 1, 1987.
tobacco auxin-inducible gene	Van der Zaal et al., Plant Mol. Biol. 16, 983, 1991.
β -tubulin	Oppenheimer, et al., Gene 63: 87, 1988.
tobacco root-specific genes	Conkling, et al., Plant Physiol. 93: 1203, 1990.
B. napus G1-3b gene	United States Patent No. 5, 401, 836
SbPRP1	Suzuki et al., Plant Mol. Biol. 21: 109-119, 1993.
LRX1	Baumberger et al. 2001, Genes & Dev. 15:1128
BTG-26 Brassica napus	US 20050044585
LeAMT1 (tomato)	Lauter et al. (1996, PNAS 3:8139)
The LeNRT1-1 (tomato)	Lauter et al. (1996, PNAS 3:8139)
class I patatin gene (potato)	Liu et al., Plant Mol. Biol. 153:386-395, 1991.
KDC1 (Daucus carota)	Downey et al. (2000, J. Biol. Chem. 275:39420)
TobRB7 gene	W Song (1997) PhD Thesis, North Carolina State University, Raleigh, NC USA
OsRAB5a (rice)	Wang et al. 2002, Plant Sci. 163:273
ALF5 (Arabidopsis)	Diener et al. (2001, Plant Cell 13:1625)
NRT2;1Np (N. plumbaginifolia)	Quesada et al. (1997, Plant Mol. Biol. 34:265)
Barley root-specific lectin	Lerner & Raikhel (1989) Plant Phys 91: 124-129
Root-specific hydroxy-proline rich protein	Keller & Lamb (1989) Genes & Dev 3:1639-1646
Arabidopsis CDC27B/hobbit	Blilou et al. (2002) Genes & Dev 16:2566-2575

[0057] A seed-specific promoter is transcriptionally active predominantly in seed tissue, but not necessarily exclusively in seed tissue (in cases of leaky expression). The seed-specific promoter may be active during seed development and/or during germination. Examples of seed-specific promoters are shown in Table 2c below. Further examples of seed-specific promoters are given in Qing Qu and Takaiwa (Plant Biotechnol. J. 2, 113-125, 2004), which disclosure is incorporated by reference herein as if fully set forth.

Table 2c: Examples of seed-specific promoters

Gene source	Reference
seed-specific genes	Simon et al., Plant Mol. Biol. 5:191, 1985;
	Scofield et al., J. Biol. Chem. 262: 12202, 1987.;
	Baszczynski et al., Plant Mol. Biol. 14: 633, 1990.
Brazil Nut albumin	Pearson et al., Plant Mol. Biol. 18: 235-245, 1992.
Legumin	Ellis et al., Plant Mol. Biol. 10: 203-214, 1988.
glutelin (rice)	Takaiwa et al., Mol. Gen. Genet. 208: 15-22, 1986;
	Takaiwa et al., FEBS Letts. 221: 43-47, 1987.

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(continued)

Gene source	Reference
Zein	Matzke et al Plant Mol Biol, 14(3):323-32 1990
NapA	Stalberg et al, Planta 199: 515-519, 1996.
Wheat LMW and HMW glutenin-1	Mol Gen Genet 216:81-90, 1989; NAR 17:461-2, 1989
Wheat SPA	Albani et al, Plant Cell, 9: 171-184, 1997
Wheat α , β , γ -gliadins	EMBO J. 3:1409-15, 1984
Barley ltr1 promoter	Diaz et al. (1995) Mol Gen Genet 248(5):592-8
Barley B1, C, D, hordein	Theor Appl Gen 98:1253-62, 1999; Plant J 4:343-55, 1993; Mol Gen Genet 250:750-60, 1996
Barley DOF	Mena et al, The Plant Journal, 116(1): 53-62, 1998
blz2	EP99106056.7
Synthetic promoter	Vicente-Carbajosa et al., Plant J. 13: 629-640, 1998.
rice prolamin NRP33	Wu et al, Plant Cell Physiology 39(8) 885-889, 1998
rice α -globulin Glb-1	Wu et al, Plant Cell Physiology 39(8) 885-889, 1998
rice OSH1	Sato et al, Proc. Natl. Acad. Sci. USA, 93: 8117-8122, 1996
rice α -globulin REB/OHP-1	Nakase et al. Plant Mol. Biol. 33: 513-522, 1997
rice ADP-glucose pyrophosphorylase	Trans Res 6:157-68, 1997
Maize ESR gene family	Plant J 12:235-46, 1997
Sorghum α -kafirin	DeRose et al., Plant Mol. Biol 32:1029-35, 1996
KNOX	Postma-Haarsma et al, Plant Mol. Biol. 39:257-71, 1999
rice oleosin	Wu et al, J. Biochem. 123:386, 1998
sunflower oleosin	Cummins et al., Plant Mol. Biol. 19: 873-876, 1992
PRO0117, putative rice 40S ribosomal protein	WO 2004/070039
PRO0136, rice alanine aminotransferase	Unpublished
PRO0147, trypsin inhibitor ITR1 (barley)	Unpublished
PRO0151, rice WSI18	WO 2004/070039
PRO0175, rice RAB21	WO 2004/070039
PRO005	WO 2004/070039
PRO0095	WO 2004/070039
α -amylase (Amy32b)	Lanahan et al, Plant Cell 4:203-211, 1992; Skriver et al, Proc Natl Acad Sci USA 88:7266-7270, 1991
Cathepsin β -like gene	Cejudo et al, Plant Mol Biol 20:849-856, 1992
Barley Ltp2	Kalla et al., Plant J. 6:849-60, 1994
Chi26	Leah et al., Plant J. 4:579-89, 1994
Maize B-Peru	Selinger et al., Genetics 149:1125-38, 1998

[0058] A "promoter active in above ground parts" refers to a promoter that is capable of preferentially initiating transcription in above ground parts of a plant substantially to the exclusion of any other parts of a plant (specifically below-ground parts), whilst still allowing for any leaky expression in these other plant parts. Table 2d below shows examples

of such promoters, which are transcriptionally active predominantly in green tissue.

[0059] A green tissue-specific promoter as defined herein is a promoter that is transcriptionally active predominantly in green tissue, substantially to the exclusion of any other parts of a plant, whilst still allowing for any leaky expression in these other plant parts.

[0060] Examples of green tissue-specific promoters which may be used to perform the methods of the invention are shown in Table 2d below.

Table 2d: Examples of green tissue-specific promoters

Gene	Expression	Reference
Maize Orthophosphate dikinase	Leaf specific	Fukavama et al., 2001
Maize Phosphoenolpyruvate carboxylase	Leaf specific	Kausch et al., 2001
Rice Phosphoenolpyruvate carboxylase	Leaf specific	Liu et al., 2003
Rice small subunit Rubisco	Leaf specific	Nomura et al., 2000
rice beta expansin EXBP9	Shoot specific	WO 2004/070039
Pigeonpea small subunit Rubisco	Leaf specific	Panguluri et al., 2005
Pea RBCS3A	Leaf specific	

[0061] Another example of a tissue-specific promoter is a meristem-specific promoter, which is transcriptionally active predominantly in meristematic tissue, substantially to the exclusion of any other parts of a plant, whilst still allowing for any leaky expression in these other plant parts. Examples of meristem-specific promoters which may be used to perform the methods of the invention are shown in Table 2e below.

Table 2e: Examples of meristem-specific promoters

Gene source	Expression pattern	Reference
rice OSH1	Shoot apical meristem, from embryo globular stage to seedling stage	Sato et al. (1996) Proc. Natl. Acad. Sci. USA, 93: 8117-8122
Rice metallothionein	Meristem specific	BAD87835.1
WAK1 & WAK 2	Shoot and root apical meristems, and in expanding leaves and sepals	Wagner & Kohorn (2001) Plant Cell 13(2): 303-318

Table 2f: examples of endosperm-specific promoters

Gene source	Reference
glutelin (rice)	Takaiwa et al. (1986) Mol Gen Genet 208:15-22; Takaiwa et al. (1987) FEBS Letts. 221:43-47
zein	Matzke et al., (1990) Plant Mol Biol 14(3): 323-32
wheat LMW and HMW glutenin-1	Colot et al. (1989) Mol Gen Genet 216:81-90, Anderson et al. (1989) NAR 17:461-2
wheat SPA	Albani et al. (1997) Plant Cell 9:171-184
wheat gliadins	Rafalski et al. (1984) EMBO 3:1409-15
barley ltr1 promoter	Diaz et al. (1995) Mol Gen Genet 248(5):592-8
barley B1, C, D, hordein	Cho et al. (1999) Theor Appl Genet 98:1253-62; Muller et al. (1993) Plant J 4:343-55; Sorenson et al. (1996) Mol Gen Genet 250:750-60

(continued)

Gene source	Reference
barley DOF	Mena et al, (1998) Plant J 116(1): 53-62
blz2	Onate et al. (1999) J Biol Chem 274(14):9175-82
synthetic promoter	Vicente-Carbajosa et al. (1998) Plant J 13:629-640
rice prolamin NRP33	Wu et al, (1998) Plant Cell Physiol 39(8) 885-889
rice globulin Glb-1	Wu et al. (1998) Plant Cell Physiol 39(8) 885-889
rice globulin REB/OHP-1	Nakase et al. (1997) Plant Molec Biol 33: 513-522
rice ADP-glucose pyrophosphorylase	Russell et al. (1997) Trans Res 6:157-68
maize ESR gene family	Opsahl-Ferstad et al. (1997) Plant J 12:235-46
sorghum kafirin	DeRose et al. (1996) Plant Mol Biol 32:1029-35

Table 2g: Examples of embryo specific promoters:

Gene source	Reference
rice OSH1	Sato et al, Proc. Natl. Acad. Sci. USA, 93: 8117-8122, 1996
KNOX	Postma-Haarsma et al, Plant Mol. Biol. 39:257-71, 1999
PRO0151	WO 2004/070039
PRO0175	WO 2004/070039
PRO005	WO 2004/070039
PRO0095	WO 2004/070039

Table 2h: Examples of aleurone-specific promoters:

Gene source	Reference
α -amylase (Amy32b)	Lanahan et al, Plant Cell 4:203-211, 1992; Skriver et al, Proc Natl Acad Sci USA 88:7266-7270, 1991
cathepsin β -like gene	Cejudo et al, Plant Mol Biol 20:849-856, 1992
Barley Ltp2	Kalla et al., Plant J. 6:849-60, 1994
Chi26	Leah et al., Plant J. 4:579-89, 1994
Maize B-Peru	Selinger et al., Genetics 149:1125-38, 1998

Terminator

[0062] The term "terminator" encompasses a control sequence which is a DNA sequence at the end of a transcriptional unit which signals 3' processing and polyadenylation of a primary transcript and termination of transcription. The terminator can be derived from the natural gene, from a variety of other plant genes, or from T-DNA. The terminator to be added

may be derived from, for example, the nopaline synthase or octopine synthase genes, or alternatively from another plant gene, or less preferably from any other eukaryotic gene.

Modulation

[0063] The term "modulation" means in relation to expression or gene expression, a process in which the expression level is changed by said gene expression in comparison to the control plant, preferably the expression level is increased. The original, unmodulated expression may be of any kind of expression of a structural RNA (rRNA, tRNA) or mRNA with subsequent translation. The term "modulating the activity" shall mean any change of the expression of the inventive nucleic acid sequences or encoded proteins, which leads to increased yield and/or increased growth of the plants.

Expression

[0064] The term "expression" or "gene expression" means the transcription of a specific gene or specific genes or specific genetic construct. The term "expression" or "gene expression" in particular means the transcription of a gene or genes or genetic construct into structural RNA (rRNA, tRNA) or mRNA with or without subsequent translation of the latter into a protein. The process includes transcription of DNA and processing of the resulting mRNA product.

Increased expression/overexpression

[0065] The term "increased expression" or "overexpression" as used herein means any form of expression that is additional to the original wild-type expression level.

[0066] Methods for increasing expression of genes or gene products are well documented in the art and include, for example, overexpression driven by appropriate promoters, the use of transcription increasers or translation increasers. Isolated nucleic acid sequences which serve as promoter or increaser elements may be introduced in an appropriate position (typically upstream) of a non-heterologous form of a polynucleotide so as to upregulate expression of a nucleic acid sequence encoding the polypeptide of interest. For example, endogenous promoters may be altered in vivo by mutation, deletion, and/or substitution (see, Kmiec, US 5,565,350; Zarling et al., WO9322443), or isolated promoters may be introduced into a plant cell in the proper orientation and distance from a gene of the present invention so as to control the expression of the gene.

[0067] If polypeptide expression is desired, it is generally desirable to include a polyadenylation region at the 3'-end of a polynucleotide coding region. The polyadenylation region can be derived from the natural gene, from a variety of other plant genes, or from T-DNA. The 3' end sequence to be added may be derived from, for example, the nopaline synthase or octopine synthase genes, or alternatively from another plant gene, or less preferably from any other eukaryotic gene.

[0068] An intron sequence may also be added to the 5' untranslated region (UTR) or the coding sequence of the partial coding sequence to increase the amount of the mature message that accumulates in the cytosol. Inclusion of a spliceable intron in the transcription unit in both plant and animal expression constructs has been shown to increase gene expression at both the mRNA and protein levels up to 1000-fold (Buchman and Berg (1988) Mol. Cell Biol. 8: 4395-4405; Callis et al. (1987) Genes Dev 1:1183-1200). Such intron increase of gene expression is typically greatest when placed near the 5' end of the transcription unit. Use of the maize introns Adh1-S intron 1, 2, and 6, the Bronze-1 intron are known in the art. For general information see: The Maize Handbook, Chapter 116, Freeling and Walbot, Eds., Springer, N.Y. (1994).

Endogenous gene

[0069] Reference herein to an "endogenous" gene not only refers to the gene in question as found in a plant in its natural form (i.e., without there being any human intervention), but also refers to that same gene (or a substantially homologous nucleic acid/gene) in an isolated form subsequently (re)introduced into a plant (a transgene). For example, a transgenic plant containing such a transgene may encounter a substantial reduction of the transgene expression and/or substantial reduction of expression of the endogenous gene.

[0070] The isolated gene may be isolated from an organism or may be manmade, for example by chemical synthesis.

Decreased expression

[0071] Reference herein to "decreased expression" or "reduction or substantial elimination" of expression is taken to mean a decrease in endogenous gene expression and/or polypeptide levels and/or polypeptide activity relative to control plants. The reduction or substantial elimination is in increasing order of preference at least 10%, 20%, 30%, 40% or

50%, 60%, 70%, 80%, 85%, 90%, or 95%, 96%, 97%, 98%, 99% or more reduced compared to that of control plants.

[0072] For the reduction or substantial elimination of expression an endogenous gene in a plant, a sufficient length of substantially contiguous nucleotides of a nucleic acid sequence is required. In order to perform gene silencing, this may be as little as 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10 or fewer nucleotides, alternatively this may be as much as the entire gene (including the 5' and/or 3' UTR, either in part or in whole). The stretch of substantially contiguous nucleotides may be derived from the nucleic acid sequence encoding the protein of interest (target gene), or from any nucleic acid sequence capable of encoding an orthologue, paralogue or homologue of the protein of interest. Preferably, the stretch of substantially contiguous nucleotides is capable of forming hydrogen bonds with the target gene (either sense or antisense strand), more preferably, the stretch of substantially contiguous nucleotides has, in increasing order of preference, 50%, 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 100% sequence identity to the target gene (either sense or antisense strand). A nucleic acid sequence encoding a (functional) polypeptide is not a requirement for the various methods discussed herein for the reduction or substantial elimination of expression of an endogenous gene.

[0073] This reduction or substantial elimination of expression may be achieved using routine tools and techniques. A method for the reduction or substantial elimination of endogenous gene expression is by RNA-mediated silencing using an inverted repeat of a nucleic acid sequence or a part thereof (in this case a stretch of substantially contiguous nucleotides derived from the gene of interest, or from any nucleic acid sequence capable of encoding an orthologue, paralogue or homologue of the protein of interest), preferably capable of forming a hairpin structure. Another example of an RNA silencing method involves the introduction of nucleic acid sequences or parts thereof (in this case a stretch of substantially contiguous nucleotides derived from the gene of interest, or from any nucleic acid sequence capable of encoding an orthologue, paralogue or homologue of the protein of interest) in a sense orientation into a plant. Another example of an RNA silencing method involves the use of antisense nucleic acid sequences. Gene silencing may also be achieved by insertion mutagenesis (for example, T-DNA insertion or transposon insertion) or by strategies as described by, among others, Angell and Baulcombe ((1999) *Plant J* 20(3): 357-62), (Amplicon VIGS WO 98/36083), or Baulcombe (WO 99/15682). Other methods, such as the use of antibodies directed to an endogenous polypeptide for inhibiting its function in planta, or interference in the signalling pathway in which a polypeptide is involved, will be well known to the skilled man. Artificial and/or natural microRNAs (miRNAs) may be used to knock out gene expression and/or mRNA translation. Endogenous miRNAs are single stranded small RNAs of typically 19-24 nucleotides long. Artificial microRNAs (amiRNAs), which are typically 21 nucleotides in length, can be genetically engineered specifically to negatively regulate gene expression of single or multiple genes of interest. Determinants of plant microRNA target selection are well known in the art. Empirical parameters for target recognition have been defined and can be used to aid in the design of specific amiRNAs (Schwab et al., (2005) *Dev Cell* 8(4):517-27). Convenient tools for design and generation of amiRNAs and their precursors are also available to the public (Schwab et al., (2006) *Plant Cell* 18(5):1121-33).

More detailed:

[0074] This reduction or substantial elimination of expression may be achieved using routine tools and techniques. A preferred method for the reduction or substantial elimination of endogenous gene expression is by introducing and expressing in a plant a genetic construct into which the nucleic acid (in this case a stretch of substantially contiguous nucleotides derived from the gene of interest, or from any nucleic acid capable of encoding an orthologue, paralogue or homologue of any one of the protein of interest) is cloned as an inverted repeat (in part or completely), separated by a spacer (non-coding DNA).

[0075] In such a preferred method, expression of the endogenous gene is reduced or substantially eliminated through RNA-mediated silencing using an inverted repeat of a nucleic acid or a part thereof (in this case a stretch of substantially contiguous nucleotides derived from the gene of interest, or from any nucleic acid capable of encoding an orthologue, paralogue or homologue of the protein of interest), preferably capable of forming a hairpin structure. The inverted repeat is cloned in an expression vector comprising control sequences. A non-coding DNA nucleic acid sequence (a spacer, for example a matrix attachment region fragment (MAR), an intron, a polylinker, etc.) is located between the two inverted nucleic acids forming the inverted repeat. After transcription of the inverted repeat, a chimeric RNA with a self-complementary structure is formed (partial or complete). This double-stranded RNA structure is referred to as the hairpin RNA (hpRNA). The hpRNA is processed by the plant into siRNAs that are incorporated into an RNA-induced silencing complex (RISC). The RISC further cleaves the mRNA transcripts, thereby substantially reducing the number of mRNA transcripts to be translated into polypeptides. For further general details see for example, Grierson et al. (1998) WO 98/53083; Waterhouse et al. (1999) WO 99/53050).

[0076] Performance of the methods of the invention does not rely on introducing and expressing in a plant a genetic construct into which the nucleic acid is cloned as an inverted repeat, but any one or more of several well-known "gene silencing" methods may be used to achieve the same effects.

[0077] One such method for the reduction of endogenous gene expression is RNA-mediated silencing of gene expression (downregulation). Silencing in this case is triggered in a plant by a double stranded RNA sequence (dsRNA)

that is substantially similar to the target endogenous gene. This dsRNA is further processed by the plant into about 20 to about 26 nucleotides called short interfering RNAs (siRNAs). The siRNAs are incorporated into an RNA-induced silencing complex (RISC) that cleaves the mRNA transcript of the endogenous target gene, thereby substantially reducing the number of mRNA transcripts to be translated into a polypeptide. Preferably, the double stranded RNA sequence corresponds to a target gene.

[0078] Another example of an RNA silencing method involves the introduction of nucleic acid sequences or parts thereof (in this case a stretch of substantially contiguous nucleotides derived from the gene of interest, or from any nucleic acid capable of encoding an orthologue, paralogue or homologue of the protein of interest) in a sense orientation into a plant. "Sense orientation" refers to a DNA sequence that is homologous to an mRNA transcript thereof. Introduced into a plant would therefore be at least one copy of the nucleic acid sequence. The additional nucleic acid sequence will reduce expression of the endogenous gene, giving rise to a phenomenon known as co-suppression. The reduction of gene expression will be more pronounced if several additional copies of a nucleic acid sequence are introduced into the plant, as there is a positive correlation between high transcript levels and the triggering of co-suppression.

[0079] Another example of an RNA silencing method involves the use of antisense nucleic acid sequences. An "antisense" nucleic acid sequence comprises a nucleotide sequence that is complementary to a "sense" nucleic acid sequence encoding a protein, i.e. complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA transcript sequence. The antisense nucleic acid sequence is preferably complementary to the endogenous gene to be silenced. The complementarity may be located in the "coding region" and/or in the "non-coding region" of a gene. The term "coding region" refers to a region of the nucleotide sequence comprising codons that are translated into amino acid residues. The term "non-coding region" refers to 5' and 3' sequences that flank the coding region that are transcribed but not translated into amino acids (also referred to as 5' and 3' untranslated regions).

[0080] Antisense nucleic acid sequences can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid sequence may be complementary to the entire nucleic acid sequence (in this case a stretch of substantially contiguous nucleotides derived from the gene of interest, or from any nucleic acid capable of encoding an orthologue, paralogue or homologue of the protein of interest), but may also be an oligonucleotide that is antisense to only a part of the nucleic acid sequence (including the mRNA 5' and 3' UTR). For example, the antisense oligonucleotide sequence may be complementary to the region surrounding the translation start site of an mRNA transcript encoding a polypeptide. The length of a suitable antisense oligonucleotide sequence is known in the art and may start from about 50, 45, 40, 35, 30, 25, 20, 15 or 10 nucleotides in length or less. An antisense nucleic acid sequence according to the invention may be constructed using chemical synthesis and enzymatic ligation reactions using methods known in the art. For example, an antisense nucleic acid sequence (e.g., an antisense oligonucleotide sequence) may be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acid sequences, e.g., phosphorothioate derivatives and acridine substituted nucleotides may be used. Examples of modified nucleotides that may be used to generate the antisense nucleic acid sequences are well known in the art. Known nucleotide modifications include methylation, cyclization and 'caps' and substitution of one or more of the naturally occurring nucleotides with an analogue such as inosine. Other modifications of nucleotides are well known in the art.

[0081] The antisense nucleic acid sequence can be produced biologically using an expression vector into which a nucleic acid sequence has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest). Preferably, production of antisense nucleic acid sequences in plants occurs by means of a stably integrated nucleic acid construct comprising a promoter, an operably linked antisense oligonucleotide, and a terminator.

[0082] The nucleic acid molecules used for silencing in the methods of the invention (whether introduced into a plant or generated in situ) hybridize with or bind to mRNA transcripts and/or genomic DNA encoding a polypeptide to thereby inhibit expression of the protein, e.g., by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid sequence which binds to DNA duplexes, through specific interactions in the major groove of the double helix. Antisense nucleic acid sequences may be introduced into a plant by transformation or direct injection at a specific tissue site. Alternatively, antisense nucleic acid sequences can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense nucleic acid sequences can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, e.g., by linking the antisense nucleic acid sequence to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid sequences can also be delivered to cells using the vectors described herein.

[0083] According to a further aspect, the antisense nucleic acid sequence is an a-anomeric nucleic acid sequence. An a-anomeric nucleic acid sequence forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual b-units, the strands run parallel to each other (Gaultier et al. (1987) Nucl Ac Res 15: 6625-6641). The antisense nucleic acid sequence may also comprise a 2'-o-methylribonucleotide (Inoue et al. (1987) Nucl Ac Res 15, 6131-6148) or a chimeric RNA-DNA analogue (Inoue et al. (1987) FEBS Lett. 215, 327-330).

[0084] The reduction or substantial elimination of endogenous gene expression may also be performed using ribozymes. Ribozymes are catalytic RNA molecules with ribonuclease activity that are capable of cleaving a single-stranded nucleic acid sequence, such as an mRNA, to which they have a complementary region. Thus, ribozymes (e.g., hammerhead ribozymes (described in Haselhoff and Gerlach (1988) *Nature* 334, 585-591) can be used to catalytically cleave mRNA transcripts encoding a polypeptide, thereby substantially reducing the number of mRNA transcripts to be translated into a polypeptide. A ribozyme having specificity for a nucleic acid sequence can be designed (see for example: Cech et al. U.S. Patent No. 4,987,071; and Cech et al. U.S. Patent No. 5,116,742). Alternatively, mRNA transcripts corresponding to a nucleic acid sequence can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules (Bartel and Szostak (1993) *Science* 261, 1411-1418). The use of ribozymes for gene silencing in plants is known in the art (e.g., Atkins et al. (1994) WO 94/00012; Lenne et al. (1995) WO 95/03404; Lutziger et al. (2000) WO 00/00619; Prinsen et al. (1997) WO 97/13865 and Scott et al. (1997) WO 97/38116).

[0085] Gene silencing may also be achieved by insertion mutagenesis (for example, T-DNA insertion or transposon insertion) or by strategies as described by, among others, Angell and Baulcombe ((1999) *Plant J* 20(3): 357-62), (Amplicon VIGS WO 98/36083), or Baulcombe (WO 99/15682).

[0086] Gene silencing may also occur if there is a mutation on an endogenous gene and/or a mutation on an isolated gene/nucleic acid subsequently introduced into a plant. The reduction or substantial elimination may be caused by a non-functional polypeptide. For example, a polypeptide may bind to various interacting proteins; one or more mutation(s) and/or truncation(s) may therefore provide for a polypeptide that is still able to bind interacting proteins (such as receptor proteins) but that cannot exhibit its normal function (such as signalling ligand).

[0087] A further approach to gene silencing is by targeting nucleic acid sequences complementary to the regulatory region of the gene (e.g., the promoter and/or enhancers) to form triple helical structures that prevent transcription of the gene in target cells. See Helene, C., *Anticancer Drug Res.* 6, 569-84, 1991; Helene et al., *Ann. N.Y. Acad. Sci.* 660, 27-36 1992; and Maher, L.J. *Bioassays* 14, 807-15, 1992.

[0088] Other methods, such as the use of antibodies directed to an endogenous polypeptide for inhibiting its function in planta, or interference in the signalling pathway in which a polypeptide is involved, will be well known to the skilled man. In particular, it can be envisaged that manmade molecules may be useful for inhibiting the biological function of a target polypeptide, or for interfering with the signalling pathway in which the target polypeptide is involved.

[0089] Alternatively, a screening program may be set up to identify in a plant population natural variants of a gene, which variants encode polypeptides with reduced activity. Such natural variants may also be used for example, to perform homologous recombination.

[0090] Artificial and/or natural microRNAs (miRNAs) may be used to knock out gene expression and/or mRNA translation. Endogenous miRNAs are single stranded small RNAs of typically 19-24 nucleotides long. They function primarily to regulate gene expression and/ or mRNA translation. Most plant microRNAs (miRNAs) have perfect or near-perfect complementarity with their target sequences. However, there are natural targets with up to five mismatches. They are processed from longer non-coding RNAs with characteristic fold-back structures by double-strand specific RNases of the Dicer family. Upon processing, they are incorporated in the RNA-induced silencing complex (RISC) by binding to its main component, an Argonaute protein. MiRNAs serve as the specificity components of RISC, since they base-pair to target nucleic acids, mostly mRNAs, in the cytoplasm. Subsequent regulatory events include target mRNA cleavage and destruction and/or translational inhibition. Effects of miRNA overexpression are thus often reflected in decreased mRNA levels of target genes.

[0091] Artificial microRNAs (amiRNAs), which are typically 21 nucleotides in length, can be genetically engineered specifically to negatively regulate gene expression of single or multiple genes of interest. Determinants of plant microRNA target selection are well known in the art. Empirical parameters for target recognition have been defined and can be used to aid in the design of specific amiRNAs, (Schwab et al., *Dev. Cell* 8, 517-527, 2005). Convenient tools for design and generation of amiRNAs and their precursors are also available to the public (Schwab et al., *Plant Cell* 18, 1121-1133, 2006).

[0092] For optimal performance, the gene silencing techniques used for reducing expression in a plant of an endogenous gene requires the use of nucleic acid sequences from monocotyledonous plants for transformation of monocotyledonous plants, and from dicotyledonous plants for transformation of dicotyledonous plants. Preferably, a nucleic acid sequence from any given plant species is introduced into that same species. For example, a nucleic acid sequence from rice is transformed into a rice plant. However, it is not an absolute requirement that the nucleic acid sequence to be introduced originates from the same plant species as the plant in which it will be introduced. It is sufficient that there is substantial homology between the endogenous target gene and the nucleic acid sequence to be introduced.

[0093] Described above are examples of various methods for the reduction or substantial elimination of expression in a plant of an endogenous gene. A person skilled in the art would readily be able to adapt the aforementioned methods for silencing so as to achieve reduction of expression of an endogenous gene in a whole plant or in parts thereof through the use of an appropriate promoter, for example.

Selectable marker (gene)/Reporter gene

[0094] "Selectable marker", "selectable marker gene" or "reporter gene" includes any gene that confers a phenotype on a cell in which it is expressed to facilitate the identification and/or selection of cells that are transfected or transformed with a nucleic acid sequence construct of the invention. These marker genes enable the identification of a successful transfer of the nucleic acid sequence molecules via a series of different principles. Suitable markers may be selected from markers that confer antibiotic or herbicide resistance, that introduce a new metabolic trait or that allow visual selection. Examples of selectable marker genes include genes conferring resistance to antibiotics (such as nptII that phosphorylates neomycin and kanamycin, or hpt, phosphorylating hygromycin, or genes conferring resistance to, for example, bleomycin, streptomycin, tetracyclin, chloramphenicol, ampicillin, gentamycin, geneticin (G418), spectinomycin or blasticidin), to herbicides (for example bar which provides resistance to Basta®; aroA or gox providing resistance against glyphosate, or the genes conferring resistance to, for example, imidazolinone, phosphinothricin or sulfonyleurea), or genes that provide a metabolic trait (such as manA that allows plants to use mannose as sole carbon source or xylose isomerase for the utilisation of xylose, or antinutritive markers such as the resistance to 2-deoxyglucose). Expression of visual marker genes results in the formation of colour (for example β -glucuronidase, GUS or β -galactosidase with its coloured substrates, for example X-Gal), luminescence (such as the luciferin/luciferase system) or fluorescence (Green Fluorescent Protein, GFP, and derivatives thereof). This list represents only a small number of possible markers. The skilled worker is familiar with such markers. Different markers are preferred, depending on the organism and the selection method.

[0095] It is known that upon stable or transient integration of nucleic acid sequences into plant cells, only a minority of the cells takes up the foreign DNA and, if desired, integrates it into its genome, depending on the expression vector used and the transfection technique used. To identify and select these integrants, a gene coding for a selectable marker (such as the ones described above) is usually introduced into the host cells together with the gene of interest. These markers can for example be used in mutants in which these genes are not functional by, for example, deletion by conventional methods. Furthermore, nucleic acid sequence molecules encoding a selectable marker can be introduced into a host cell on the same vector that comprises the sequence encoding the polypeptides of the invention or used in the methods of the invention, or else in a separate vector. Cells which have been stably transfected with the introduced nucleic acid sequence can be identified for example by selection (for example, cells which have integrated the selectable marker survive whereas the other cells die).

[0096] Since the marker genes, particularly genes for resistance to antibiotics and herbicides, are no longer required or are undesired in the transgenic host cell once the nucleic acid sequences have been introduced successfully, the process according to the invention for introducing the nucleic acid sequences advantageously employs techniques which enable the removal or excision of these marker genes. One such a method is what is known as co-transformation. The co-transformation method employs two vectors simultaneously for the transformation, one vector bearing the nucleic acid sequence according to the invention and a second bearing the marker gene(s). A large proportion of transformants receives or, in the case of plants, comprises (up to 40% or more of the transformants), both vectors. In case of transformation with *Agrobacteria*, the transformants usually receive only a part of the vector, i.e. the sequence flanked by the T-DNA, which usually represents the expression cassette. The marker genes can subsequently be removed from the transformed plant by performing crosses. In another method, marker genes integrated into a transposon are used for the transformation together with desired nucleic acid sequence (known as the Ac/Ds technology). The transformants can be crossed with a transposase source or the transformants are transformed with a nucleic acid sequence construct conferring expression of a transposase, transiently or stable. In some cases (approx. 10%), the transposon jumps out of the genome of the host cell once transformation has taken place successfully and is lost. In a further number of cases, the transposon jumps to a different location. In these cases the marker gene must be eliminated by performing crosses. In microbiology, techniques were developed which make possible, or facilitate, the detection of such events. A further advantageous method relies on what is known as recombination systems; whose advantage is that elimination by crossing can be dispensed with. The best-known system of this type is what is known as the Cre/lox system. Cre1 is a recombinase that removes the sequences located between the loxP sequences. If the marker gene is integrated between the loxP sequences, it is removed once transformation has taken place successfully, by expression of the recombinase. Further recombination systems are the HIN/HIX, FLP/FRT and REP/STB system (Tribble et al., J. Biol. Chem., 275, 2000: 22255-22267; Velmurugan et al., J. Cell Biol., 149, 2000: 553-566). A site-specific integration into the plant genome of the nucleic acid sequences according to the invention is possible. Naturally, these methods can also be applied to microorganisms such as yeast, fungi or bacteria.

Transgenic/Transgene/Recombinant

[0097] For the purposes of the invention, "transgenic", "transgene" or "recombinant" means with regard to, for example, a nucleic acid sequence, an expression cassette, gene construct or a vector comprising the nucleic acid sequence or

an organism transformed with the nucleic acid sequences, expression cassettes or vectors according to the invention, all those constructions brought about by recombinant methods in which either

- (a) the nucleic acid sequences encoding proteins useful in the methods of the invention, or
- (b) genetic control sequence(s) which is operably linked with the nucleic acid sequence according to the invention, for example a promoter, or
- (c) a) and b)

are not located in their natural genetic environment or have been modified by recombinant methods, it being possible for the modification to take the form of, for example, a substitution, addition, deletion, inversion or insertion of one or more nucleotide residues. The natural genetic environment is understood as meaning the natural genomic or chromosomal locus in the original plant or the presence in a genomic library. In the case of a genomic library, the natural genetic environment of the nucleic acid sequence is preferably retained, at least in part. The environment flanks the nucleic acid sequence at least on one side and has a sequence length of at least 50 bp, preferably at least 500 bp, especially preferably at least 1000 bp, most preferably at least 5000 bp. A naturally occurring expression cassette - for example the naturally occurring combination of the natural promoter of the nucleic acid sequences with the corresponding nucleic acid sequence encoding a polypeptide useful in the methods of the present invention, as defined above - becomes a transgenic expression cassette when this expression cassette is modified by non-natural, synthetic ("artificial") methods such as, for example, mutagenic treatment. Suitable methods are described, for example, in US 5,565,350 or WO 00/15815.

[0098] A transgenic plant for the purposes of the invention is thus understood as meaning, as above, that the nucleic acid sequences used in the method of the invention are not at their natural locus in the genome of said plant, it being possible for the nucleic acid sequences to be expressed homologously or heterologously. However, as mentioned, transgenic also means that, while the nucleic acid sequence according to the invention or used in the inventive method are at their natural position in the genome of a plant, the sequence has been modified with regard to the natural sequence, and/or that the regulatory sequences of the natural sequences have been modified. Transgenic is preferably understood as meaning the expression of the nucleic acid sequences according to the invention at an unnatural locus in the genome, i.e. homologous or, preferably, heterologous expression of the nucleic acid sequences takes place. Preferred transgenic plants are mentioned herein.

Transformation

[0099] The term "introduction" or "transformation" as referred to herein encompasses the transfer of an exogenous polynucleotide into a host cell, irrespective of the method used for transfer. Plant tissue capable of subsequent clonal propagation, whether by organogenesis or embryogenesis, may be transformed with a genetic construct of the present invention and a whole plant regenerated there from. The particular tissue chosen will vary depending on the clonal propagation systems available for, and best suited to, the particular species being transformed. Exemplary tissue targets include leaf disks, pollen, embryos, cotyledons, hypocotyls, megagametophytes, callus tissue, existing meristematic tissue (e.g., apical meristem, axillary buds, and root meristems), and induced meristem tissue (e.g., cotyledon meristem and hypocotyl meristem). The polynucleotide may be transiently or stably introduced into a host cell and may be maintained non-integrated, for example, as a plasmid. Alternatively, it may be integrated into the host genome. The resulting transformed plant cell may then be used to regenerate a transformed plant in a manner known to persons skilled in the art.

[0100] The transfer of foreign genes into the genome of a plant is called transformation. Transformation of plant species is now a fairly routine technique. Advantageously, any of several transformation methods may be used to introduce the gene of interest into a suitable ancestor cell. The methods described for the transformation and regeneration of plants from plant tissues or plant cells may be utilized for transient or for stable transformation. Transformation methods include the use of liposomes, electroporation, chemicals that increase free DNA uptake, injection of the DNA directly into the plant, particle gun bombardment, transformation using viruses or pollen and microprojection. Methods may be selected from the calcium/polyethylene glycol method for protoplasts (Krens, F.A. et al., (1982) Nature 296, 72-74; Negrutiu I et al. (1987) Plant Mol Biol 8: 363-373); electroporation of protoplasts (Shillito R.D. et al. (1985) Bio/Technol 3, 1099-1102); microinjection into plant material (Crossway A et al., (1986) Mol. Gen Genet 202: 179-185); DNA or RNA-coated particle bombardment (Klein TM et al., (1987) Nature 327: 70) infection with (non-integrative) viruses and the like. Transgenic plants, including transgenic crop plants, are preferably produced via *Agrobacterium*-mediated transformation. An advantageous transformation method is the transformation *in planta*. To this end, it is possible, for example, to allow the agrobacteria to act on plant seeds or to inoculate the plant meristem with agrobacteria. It has proved particularly expedient in accordance with the invention to allow a suspension of transformed agrobacteria to act on the intact plant or at least on the flower primordia. The plant is subsequently grown on until the seeds of the treated plant are obtained (Clough and Bent, Plant J. (1998) 16, 735-743). Methods for *Agrobacterium*-mediated transformation of rice include well known

methods for rice transformation, such as those described in any of the following: European patent application EP 1198985 A1, Aldemita and Hodges (Planta 199: 612-617, 1996); Chan et al. (Plant Mol Biol 22 (3): 491-506, 1993), Hiei et al. (Plant J 6 (2): 271-282, 1994), which disclosures are incorporated by reference herein as if fully set forth. In the case of corn transformation, the preferred method is as described in either Ishida et al. (Nat. Biotechnol 14(6): 745-50, 1996) or Frame et al. (Plant Physiol 129(1): 13-22, 2002), which disclosures are incorporated by reference herein as if fully set forth. Said methods are further described by way of example in B. Jenes et al., Techniques for Gene Transfer, in: Transgenic Plants, Vol. 1, Engineering and Utilization, eds. S.D. Kung and R. Wu, Academic Press (1993) 128-143 and in Potrykus Annu. Rev. Plant Physiol. Plant Molec. Biol. 42 (1991) 205-225). The nucleic acid sequences or the construct to be expressed is preferably cloned into a vector, which is suitable for transforming *Agrobacterium tumefaciens*, for example pBin19 (Bevan et al., Nucl. Acids Res. 12 (1984) 8711). *Agrobacteria* transformed by such a vector can then be used in known manner for the transformation of plants, such as plants used as a model, like *Arabidopsis thaliana* is within the scope of the present invention not considered as a crop plant), or crop plants such as, by way of example, tobacco plants, for example by immersing bruised leaves or chopped leaves in an agrobacterial solution and then culturing them in suitable media. The transformation of plants by means of *Agrobacterium tumefaciens* is described, for example, by Höfgen and Willmitzer in Nucl. Acid Res. (1988) 16, 9877 or is known inter alia from F.F. White, Vectors for Gene Transfer in Higher Plants; in Transgenic Plants, Vol. 1, Engineering and Utilization, eds. S.D. Kung and R. Wu, Academic Press, 1993, pp. 15-38.

[0101] In addition to the transformation of somatic cells, which then have to be regenerated into intact plants, it is also possible to transform the cells of plant meristems and in particular those cells which develop into gametes. In this case, the transformed gametes follow the natural plant development, giving rise to transgenic plants. Thus, for example, seeds of *Arabidopsis* are treated with agrobacteria and seeds are obtained from the developing plants of which a certain proportion is transformed and thus transgenic [Feldman, KA and Marks MD (1987). Mol Gen Genet 208:274-289; Feldmann K (1992). In: C Koncz, N-H Chua and J Shell, eds, Methods in Arabidopsis Research. Word Scientific, Singapore, pp. 274-289]. Alternative methods are based on the repeated removal of the inflorescences and incubation of the excision site in the center of the rosette with transformed agrobacteria, whereby transformed seeds can likewise be obtained at a later point in time (Chang (1994). Plant J. 5: 551-558; Katavic (1994). Mol Gen Genet, 245: 363-370). However, an especially effective method is the vacuum infiltration method with its modifications such as the "floral dip" method. In the case of vacuum infiltration of *Arabidopsis*, intact plants under reduced pressure are treated with an agrobacterial suspension [Bechthold, N (1993). C R Acad Sci Paris Life Sci, 316: 1194-1199], while in the case of the "floral dip" method the developing floral tissue is incubated briefly with a surfactant-treated agrobacterial suspension [Clough, SJ and Bent AF (1998) The Plant J. 16, 735-743]. A certain proportion of transgenic seeds are harvested in both cases, and these seeds can be distinguished from non-transgenic seeds by growing under the above-described selective conditions. In addition the stable transformation of plastids is of advantages because plastids are inherited maternally in most crops reducing or eliminating the risk of transgene flow through pollen. The transformation of the chloroplast genome is generally achieved by a process which has been schematically displayed in Klaus et al., 2004 [Nature Biotechnology 22 (2), 225-229]. Briefly the sequences to be transformed are cloned together with a selectable marker gene between flanking sequences homologous to the chloroplast genome. These homologous flanking sequences direct site specific integration into the plastome. Plastidal transformation has been described for many different plant species and an overview is given in Bock (2001) Transgenic plastids in basic research and plant biotechnology. J Mol Biol. 2001 Sep 21; 312 (3):425-38 or Maliga, P (2003) Progress towards commercialization of plastid transformation technology. Trends Biotechnol. 21, 20-28. Further biotechnological progress has recently been reported in form of marker free plastid transformants, which can be produced by a transient co-integrated marker gene (Klaus et al., 2004, Nature Biotechnology 22(2), 225-229).

T-DNA activation tagging

[0102] T-DNA activation tagging (Hayashi et al. Science (1992) 1350-1353), involves insertion of T-DNA, usually containing a promoter (may also be a translation increaser or an intron), in the genomic region of the gene of interest or 10 kb up- or downstream of the coding region of a gene in a configuration such that the promoter directs expression of the targeted gene. Typically, regulation of expression of the targeted gene by its natural promoter is disrupted and the gene falls under the control of the newly introduced promoter. The promoter is typically embedded in a T-DNA. This T-DNA is randomly inserted into the plant genome, for example, through *Agrobacterium* infection and leads to modified expression of genes near the inserted T-DNA. The resulting transgenic plants show dominant phenotypes due to modified expression of genes close to the introduced promoter.

TILLING

[0103] The term "TILLING" is an abbreviation of "Targeted Induced Local Lesions In Genomes" and refers to a mu-

tagenesis technology useful to generate and/or identify nucleic acid sequences encoding proteins with modified expression and/or activity. TILLING also allows selection of plants carrying such mutant variants. These mutant variants may exhibit modified expression, either in strength or in location or in timing (if the mutations affect the promoter for example). These mutant variants may exhibit higher activity than that exhibited by the gene in its natural form. TILLING combines high-density mutagenesis with high-throughput screening methods. The steps typically followed in TILLING are: (a) EMS mutagenesis (Redei GP and Koncz C (1992) In *Methods in Arabidopsis Research*, Koncz C, Chua NH, Schell J, eds. Singapore, World Scientific Publishing Co, pp. 16-82; Feldmann et al., (1994) In Meyerowitz EM, Somerville CR, eds, *Arabidopsis*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp 137-172; Lightner J and Caspar T (1998) In J Martinez-Zapater, J Salinas, eds, *Methods on Molecular Biology*, Vol. 82. Humana Press, Totowa, NJ, pp 91-104); (b) DNA preparation and pooling of individuals; (c) PCR amplification of a region of interest; (d) denaturation and annealing to allow formation of heteroduplexes; (e) DHPLC, where the presence of a heteroduplex in a pool is detected as an extra peak in the chromatogram; (f) identification of the mutant individual; and (g) sequencing of the mutant PCR product. Methods for TILLING are well known in the art (McCallum et al., (2000) *Nat Biotechnol* 18: 455-457; reviewed by Stemple (2004) *Nat Rev Genet* 5(2): 145-50).

Homologous recombination

[0104] Homologous recombination allows introduction in a genome of a selected nucleic acid sequence at a defined selected position. Homologous recombination is a standard technology used routinely in biological sciences for lower organisms such as yeast or the moss *Physcomitrella*. Methods for performing homologous recombination in plants have been described not only for model plants (Offringa et al. (1990) *EMBO J* 9(10): 3077-84) but also for crop plants, for example rice (Terada et al. (2002) *Nat Biotech* 20(10): 1030-4; Iida and Terada (2004) *Curr Opin Biotech* 15(2): 132-8).

Yield

[0105] The term "yield" in general means a measurable produce of economic value, typically related to a specified crop, to an area, and to a period of time. Individual plant parts directly contribute to yield based on their number, size and/or weight, or the actual yield is the yield per acre for a crop and year, which is determined by dividing total production (includes both harvested and appraised production) by planted acres. The term "yield" of a plant may relate to vegetative biomass, to reproductive organs, and/or to propagules (such as seeds) of that plant.

[0106] The term "yield" of a plant may relate to vegetative biomass (root and/or shoot biomass), to reproductive organs, and/or to propagules (such as seeds) of that plant.

Early vigour

[0107] "Early vigour" refers to active healthy well-balanced growth especially during early stages of plant growth, and may result from increased plant fitness due to, for example, the plants being better adapted to their environment (i.e. optimizing the use of energy resources and partitioning between shoot and root). Plants having early vigour also show increased seedling survival and a better establishment of the crop, which often results in highly uniform fields (with the crop growing in uniform manner, i.e. with the majority of plants reaching the various stages of development at substantially the same time), and often better and higher yield. Therefore, early vigour may be determined by measuring various factors, such as thousand kernel weight, percentage germination, percentage emergence, seedling growth, seedling height, root length, root and shoot biomass and many more.

Increase/Improve/Increase

[0108] The terms "increase", "improve" or "increase" are interchangeable and shall mean in the sense of the application at least a 5%, 6%, 7%, 8%, 9% or 10%, preferably at least 15% or 20%, more preferably 25%, 30%, 35% or 40% more yield and/or growth in comparison to control plants as defined herein.

Seed yield

[0109] Increased seed yield may manifest itself as one or more of the following: a) an increase in seed biomass (total seed weight) which may be on an individual seed basis and/or per plant and/or per hectare or acre; b) increased number of flowers per panicle and/or per plant; c) increased number of (filled) seeds; d) increased seed filling rate (which is expressed as the ratio between the number of filled seeds divided by the total number of seeds); e) increased harvest index, which is expressed as a ratio of the yield of harvestable parts, such as seeds, divided by the total biomass; f) increased number of primary panicles; (g) increased thousand kernel weight (TKW), which is extrapolated from the

number of filled seeds counted and their total weight. An increased TKW may result from an increased seed size and/or seed weight, and may also result from an increase in embryo and/or endosperm size.

[0110] An increase in seed yield may also be manifested as an increase in seed size and/or seed volume. Furthermore, an increase in seed yield may also manifest itself as an increase in seed area and/or seed length and/or seed width and/or seed perimeter. Increased yield may also result in modified architecture, or may occur because of modified architecture.

Greenness Index

[0111] The "greenness index" as used herein is calculated from digital images of plants. For each pixel belonging to the plant object on the image, the ratio of the green value versus the red value (in the RGB model for encoding color) is calculated. The greenness index is expressed as the percentage of pixels for which the green-to-red ratio exceeds a given threshold. Under normal growth conditions, under salt stress growth conditions, and under reduced nutrient availability growth conditions, the greenness index of plants is measured in the last imaging before flowering. In contrast, under drought stress growth conditions, the greenness index of plants is measured in the first imaging after drought.

Plant

[0112] The term "plant" as used herein encompasses whole plants, ancestors and progeny of the plants and plant parts, including seeds, shoots, stems, leaves, roots (including tubers), flowers, and tissues and organs, wherein each of the aforementioned comprise the gene/nucleic acid sequence of interest. The term "plant" also encompasses plant cells, suspension cultures, callus tissue, embryos, meristematic regions, gametophytes, sporophytes, pollen and microspores, again wherein each of the aforementioned comprises the gene/nucleic acid sequence of interest.

[0113] Plants that are particularly useful in the methods of the invention include all plants which belong to the superfamily Viridiplantae, in particular monocotyledonous and dicotyledonous plants including fodder or forage legumes, ornamental plants, food crops, trees or shrubs selected from the list comprising *Acer* spp., *Actinidia* spp., *Abelmoschus* spp., *Agave sisalana*, *Agropyron* spp., *Agrostis stolonifera*, *Allium* spp., *Amaranthus* spp., *Ammophila arenaria*, *Ananas comosus*, *Annona* spp., *Apium graveolens*, *Arachis* spp., *Artocarpus* spp., *Asparagus officinalis*, *Avena* spp. (e.g. *Avena sativa*, *Avena fatua*, *Avena byzantina*, *Avena fatua* var. *sativa*, *Avena hybrida*), *Averrhoa carambola*, *Bambusa* sp., *Benincasa hispida*, *Bertholletia excelsa*, *Beta vulgaris*, *Brassica* spp. (e.g. *Brassica napus*, *Brassica rapa* ssp. [canola, oilseed rape, turnip rape]), *Cadaba farinosa*, *Camellia sinensis*, *Canna indica*, *Cannabis sativa*, *Capsicum* spp., *Carex elata*, *Carica papaya*, *Carissa macrocarpa*, *Carya* spp., *Carthamus tinctorius*, *Castanea* spp., *Ceiba pentandra*, *Cichorium endivia*, *Cinnamomum* spp., *Citrullus lanatus*, *Citrus* spp., *Cocos* spp., *Coffea* spp., *Colocasia esculenta*, *Cola* spp., *Corchorus* sp., *Coriandrum safivum*, *Corylus* spp., *Crataegus* spp., *Crocus sativus*, *Cucurbita* spp., *Cucumis* spp., *Cynara* spp., *Daucus carota*, *Desmodium* spp., *Dimocarpus longan*, *Dioscorea* spp., *Diospyros* spp., *Echinochloa* spp., *Elaeis* (e.g. *Elaeis guineensis*, *Elaeis oleifera*), *Eleusine coracana*, *Erianthus* sp., *Eriobotrya japonica*, *Eucalyptus* sp., *Eugenia uniflora*, *Fagopyrum* spp., *Fagus* spp., *Festuca arundinacea*, *Ficus carica*, *Fortunella* spp., *Fragaria* spp., *Ginkgo biloba*, *Glycine* spp. (e.g. *Glycine max*, *Soja hispida* or *Soja max*), *Gossypium hirsutum*, *Helianthus* spp. (e.g. *Helianthus annuus*), *Hemerocallis fulva*, *Hibiscus* spp., *Hordeum* spp. (e.g. *Hordeum vulgare*), *Ipomoea batatas*, *Juglans* spp., *Lactuca sativa*, *Lathyrus* spp., *Lens culinaris*, *Linum usitatissimum*, *Litchi chinensis*, *Lotus* spp., *Luffa acutangula*, *Lupinus* spp., *Luzula sylvatica*, *Lycopersicon* spp. (e.g. *Lycopersicon esculentum*, *Lycopersicon lycopersicum*, *Lycopersicon pyriforme*), *Macrotyloma* spp., *Malus* spp., *Malpighia emarginata*, *Mammea americana*, *Mangifera indica*, *Manihot* spp., *Manilkara zapota*, *Medicago sativa*, *Melilotus* spp., *Mentha* spp., *Miscanthus sinensis*, *Momordica* spp., *Morus nigra*, *Musa* spp., *Nicotiana* spp., *Olea* spp., *Opuntia* spp., *Ornithopus* spp., *Oryza* spp. (e.g. *Oryza sativa*, *Oryza latifolia*), *Panicum miliaceum*, *Panicum virgatum*, *Passiflora edulis*, *Pastinaca sativa*, *Pennisetum* sp., *Persea* spp., *Petroselinum crispum*, *Phalaris arundinacea*, *Phaseolus* spp., *Phleum pratense*, *Phoenix* spp., *Phragmites australis*, *Physalis* spp., *Pinus* spp., *Pistacia vera*, *Pisum* spp., *Poa* spp., *Populus* spp., *Prosopis* spp., *Prunus* spp., *Psidium* spp., *Punica granatum*, *Pyrus communis*, *Quercus* spp., *Raphanus sativus*, *Rheum rhabarbarum*, *Ribes* spp., *Ricinus communis*, *Rubus* spp., *Saccharum* spp., *Salix* sp., *Sambucus* spp., *Secale cereale*, *Sesamum* spp., *Sinapis* sp., *Solanum* spp. (e.g. *Solanum tuberosum*, *Solanum integrifolium* or *Solanum lycopersicum*), *Sorghum bicolor*, *Spinacia* spp., *Syzygium* spp., *Tagetes* spp., *Tamarindus indica*, *Theobroma cacao*, *Trifolium* spp., *Triticale* sp., *Triticosecale rimpaui*, *Triticum* spp. (e.g. *Triticum aestivum*, *Triticum durum*, *Triticum turgidum*, *Triticum hybernum*, *Triticum macha*, *Triticum sativum* or *Triticum vulgare*), *Tropaeolum minus*, *Tropaeolum majus*, *Vaccinium* spp., *Vicia* spp., *Vigna* spp., *Viola odorata*, *Vitis* spp., *Zea mays*, *Zizania palustris*, *Ziziphus* spp., amongst others.

Detailed description of the invention

[0114] Surprisingly, it has now been found that increasing expression in a plant of a nucleic acid sequence encoding

an AHL19/20 polypeptide gives plants having increased seed yield-related traits, without delayed flowering, relative to control plants. According to a first embodiment, the present invention provides a method for increasing seed yield-related traits in plants relative to control plants, comprising increasing expression in a plant of a nucleic acid sequence encoding an AHL19/20 polypeptide.

[0115] A preferred method for increasing expression of a nucleic acid sequence encoding an AHL19/20 polypeptide is by introducing and expressing in a plant a nucleic acid sequence encoding an AHL19/20 polypeptide.

[0116] In one embodiment any reference hereinafter to a "protein useful in the methods of the invention" is taken to mean an AHL19/20 polypeptide as defined herein. Any reference hereinafter to a "nucleic acid sequence useful in the methods of the invention" is taken to mean a nucleic acid sequence capable of encoding such an AHL19/20 polypeptide. The nucleic acid sequence to be introduced into a plant (and therefore useful in performing the methods of the invention) is any nucleic acid sequence encoding the type of polypeptide, which will now be described, hereafter also named "AHL19/20 nucleic acid sequence" or "AHL19/20 gene".

[0117] An "AHL19/20 polypeptide" as defined herein refers to any polypeptide comprising a domain having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% or more amino acid sequence identity to a Conserved Domain (CD) as represented by SEQ ID NO: 36 (comprised in SEQ ID NO: 2).

[0118] Alternatively or additionally, an "AHL19/20 polypeptide" as defined herein refers to any polypeptide comprising: (i) a motif having at least 75%, 80%, 85%, 90%, 95%, 98%, 99% or more amino acid sequence identity to an AT-hook motif as represented by SEQ ID NO: 37; and (ii) a domain having at least 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% or more amino acid sequence identity to a plant and prokaryote conserved (PPC) domain as represented by SEQ ID NO: 38.

[0119] Alternatively or additionally, an "AHL19/20 polypeptide" as defined herein refers to any polypeptide comprising: (i) a nuclear localisation signal; (ii) an AT-hook DNA binding motif with an InterPro entry IPR014476; and (iii) a plant and prokaryote conserved (PPC) domain with an InterPro entry IPR005175.

[0120] Alternatively or additionally, an "AHL19/20 polypeptide" as defined herein refers to any polypeptide sequence which when used in the construction of an AHL phylogenetic tree, such as the one depicted in Figure 1 and in Figure 2, clusters with the AHL19/20 group of polypeptides comprising the polypeptide sequence as represented by SEQ ID NO: 2, rather than with any other AHL group.

[0121] Alternatively or additionally, an "AHL19/20 polypeptide" as defined herein refers to any polypeptide having in increasing order of preference at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% or more amino acid sequence identity to the AHL19/20 polypeptide as represented by SEQ ID NO: 2 or to any of the full length polypeptide sequences given in Table A herein.

[0122] It has now further been found that increasing expression in a plant of a nucleic acid sequence encoding a GRP polypeptide, wherein said GRP polypeptide is a metallothionein 2a (MT2a) polypeptide, gives plants grown under abiotic stress conditions having enhanced yield-related traits relative to control plants. According to a first embodiment, the present invention provides a method for enhancing yield-related traits in plants grown under abiotic stress conditions, relative to control plants, comprising increasing expression in a plant of a nucleic acid sequence encoding a GRP polypeptide, wherein said GRP polypeptide is a metallothionein 2a (MT2a) polypeptide.

[0123] A preferred method for increasing expression of a nucleic acid sequence encoding a GRP polypeptide is by introducing and expressing in a plant a nucleic acid sequence encoding a GRP polypeptide.

[0124] In one embodiment any reference hereinafter to a "polypeptide useful in the methods of the invention" is taken to mean a GRP polypeptide as defined herein. Any reference hereinafter to a "nucleic acid sequence useful in the methods of the invention" is taken to mean a nucleic acid sequence capable of encoding such a GRP polypeptide. The nucleic acid sequence to be introduced into a plant (and therefore useful in performing the metallothionein 2a (MT2a) polypeptide methods of the invention) is any nucleic acid sequence encoding the type of protein which will now be described, hereafter also named "GRP nucleic acid sequence" or "GRP gene".

[0125] A "GRP polypeptide" as defined herein refers the proteins represented by SEQ ID NO: 46, and to orthologues, paralogues, and homologues thereof.

[0126] Preferably, the orthologues, paralogues, and homologues of SEQ ID NO: 46 have an InterPro entry IPR000347, described as plant metallothionein, family 15.

[0127] Alternatively or additionally, a "GRP polypeptide" as defined herein refers to any polypeptide having in increasing order of preference at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% or more amino acid sequence identity to the GRP polypeptide as represented by SEQ ID NO: 46.

[0128] Metallothioneins are well known in the art, for a recent overview and classification, see Cobbett and Goldsbrough (2002). Metallothioneins are small proteins with a dumbbell conformation that finds its origin in conserved N-terminal and C-terminal cysteine rich domains which are separated from each other by a region that is variable in length and amino acid composition. Based on the primary structure 4 types of metallothioneins are discriminated. The metallothionein of SEQ ID NO: 46 comprises a conserved N-terminal domain typical for type 2 metallothioneins as defined by Cobbett and Goldsbrough (2002), which domain comprises the consensus sequence "MSC-

CGG(N/S)CGCG(T/S/A)(G/A/S)C(K/Q/S)C", accordingly, preferred homologues to be used in the methods of the present invention are metallothioneins comprising this conserved domain.

[0129] Additionally, it has now been found that preferentially modulating expression in above ground plants parts of a nucleic acid encoding an AAT-like polypeptide gives plants having enhanced yield-related traits relative to control plants. According to a preferred embodiment, preferentially modulating expression in above ground plant parts is effected through the use of a promoter active in above ground plant parts. The term "promoter active in above ground parts" is defined in the "Definitions" section herein.

[0130] Further it has now been found that modulating expression of a nucleic acid encoding an AAT polypeptide gives plants grown under non nitrogen limiting conditions enhanced yield-related traits relative to control plants. According to a first embodiment, the present invention provides a method for enhancing yield-related traits in plants relative to control plants, comprising modulating expression of a nucleic acid encoding an AAT polypeptide in plants grown under non nitrogen limiting conditions.

[0131] A preferred method for modulating (preferably, increasing) expression of a nucleic acid encoding an AAT-like polypeptide under the control of a promoter active in above ground plant parts is by introducing and expressing in a plant a nucleic acid encoding an AAT-like polypeptide under the control of a promoter active in above ground plant parts.

[0132] A preferred method for modulating (preferably, increasing) expression of a nucleic acid encoding an AAT polypeptide is by introducing and expressing in a plant a nucleic acid encoding an AAT polypeptide.

[0133] In one embodiment any reference hereinafter to a "protein useful in the methods of the invention" is taken to mean an AAT-like polypeptide as defined herein. Any reference hereinafter to a "nucleic acid useful in the methods of the invention" is taken to mean a nucleic acid capable of encoding such an AAT-like polypeptide. The nucleic acid to be introduced into a plant (and therefore useful in performing the methods of the invention) is any nucleic acid encoding the type of protein which will now be described, hereafter also named "AAT-like nucleic acid" or "AAT-like gene".

[0134] In one embodiment any reference hereinafter to a "protein useful in the methods of the invention" is taken to mean an AAT polypeptide as defined herein. Any reference hereinafter to a "nucleic acid useful in the methods of the invention" is taken to mean a nucleic acid capable of encoding such an AAT polypeptide. The nucleic acid to be introduced into a plant (and therefore useful in performing the methods of the invention) is any nucleic acid encoding the type of protein which will now be described, hereafter also named "AAT nucleic acid" or "AAT gene".

[0135] An "AAT-like polypeptide" or an "AAT polypeptide" as defined herein refers to any polypeptide having one or more of the following features:

(a) the ability to catalyse the following reaction:



(b) belongs to enzyme classification code: EC 2.6.1.2.

(c) has an amino transferase domain (referred to in InterPro by IPR004839; and in PFAM by PF00155)

(d) has an 1-aminocyclopropane-1-carboxylate synthase domain (referred to in InterPro by IPR001176)

(e) is targeted to the mitochondria

(f) when used in the construction of a phylogenetic tree containing AAT sequences, clusters with the group of AAT-like polypeptides or AAT- polypeptides comprising SEQ ID NO: 51 or SEQ ID NO: 56 rather than with any other group of AATs or AAT-like sequences.

[0136] The term "domain" and "motif" is defined in the "definitions" section herein. Specialist databases exist for the identification of domains, for example, SMART (Schultz et al. (1998) Proc. Natl. Acad. Sci. USA 95, 5857-5864; Letunic et al. (2002) Nucleic Acids Res 30, 242-244), InterPro (Mulder et al., (2003) Nucl. Acids. Res. 31, 315-318), Prosite (Bucher and Bairoch (1994), A generalized profile syntax for biomolecular sequences motifs and its function in automatic sequence interpretation. (In) ISMB-94; Proceedings 2nd International Conference on Intelligent Systems for Molecular Biology. Altman R., Brutlag D., Karp P., Lathrop R., Searls D., Eds., pp53-61, AAAI Press, Menlo Park; Hulo et al., Nucl. Acids. Res. 32: D134-D137, (2004)), or Pfam (Bateman et al., Nucleic Acids Research 30(1): 276-280 (2002)). A set of tools for *in silico* analysis of protein sequences is available on the ExPASy proteomics server (Swiss Institute of Bioinformatics (Gasteiger et al., ExPASy: the proteomics server for in-depth protein knowledge and analysis, Nucleic Acids Res. 31:3784-3788(2003)). Analysis of the polypeptide sequence of SEQ ID NO: 2 is presented below in Examples 2 and 4 herein. For example, an AHL19/20 polypeptide as represented by SEQ ID NO: 2 comprises an AT-hook DNA binding motif with an InterPro entry IPR014476, and a plant and prokaryotes conserved (PPC) domain, described as DUF296 (domain of unknown function 296) with an InterPro entry IPR005175, in the InterPro domain database. Domains may also be identified using routine techniques, such as by sequence alignment. One such domain is the Conserved Domain (CD) of SEQ ID NO: 2, as represented by SEQ ID NO: 36. The CD comprises a predicted NLS, an AT-hook DNA binding motif, and a PCC domain, as schematically represented in Figure 3, and shown in Figure 4.

[0137] Methods for the alignment of sequences for comparison are well known in the art, such methods include GAP, BESTFIT, BLAST, FASTA and TFASTA. GAP uses the algorithm of Needleman and Wunsch ((1970) J Mol Biol 48: 443-453) to find the global (i.e. spanning the complete sequences) alignment of two sequences that maximizes the number of matches and minimizes the number of gaps. The BLAST algorithm (Altschul et al. (1990) J Mol Biol 215: 403-10) calculates percent sequence identity and performs a statistical analysis of the similarity between the two sequences. The software for performing BLAST analysis is publicly available through the National Centre for Biotechnology Information (NCBI). Homologues may readily be identified using, for example, the ClustalW multiple sequence alignment algorithm (version 1.83), with the default pairwise alignment parameters, and a scoring method in percentage. Global percentages of similarity and identity may also be determined using one of the methods available in the MatGAT software package (Campanella et al., (2003) BMC Bioinformatics, 10: 29. MatGAT: an application that generates similarity/identity matrices using protein or DNA sequences.). Minor manual editing may be performed to optimise alignment between conserved motifs, as would be apparent to a person skilled in the art. Furthermore, instead of using full-length sequences for the identification of homologues, specific domains may also be used. The sequence identity values may be determined over the entire nucleic acid sequence or polypeptide sequence or over selected domains or conserved motif(s), using the programs mentioned above using the default parameters. Example 3 herein describes in Table B the percentage identity between the AHL19/20 polypeptide as represented by SEQ ID NO: 2 and the AHL19/20 polypeptides listed in Table A, which ranges between 50 and 99% amino acid sequence identity. In Table B1, the percentage identity between the CD as represented by SEQ ID NO: 36 (comprised in SEQ ID NO: 2) and the CD of the AHL19/20 polypeptides listed in Table A of Example 1 is shown, ranging from 70 to 99% amino acid sequence identity.

[0138] The task of protein subcellular localisation prediction is important and well studied. Knowing a protein's localisation helps elucidate its function. Experimental methods for protein localization range from immunolocalization to tagging of proteins using green fluorescent protein (GFP). Such methods are accurate although labor-intensive compared with computational methods. Recently much progress has been made in computational prediction of protein localisation from sequence data. Among algorithms well known to a person skilled in the art are available at the ExPASy Proteomics tools hosted by the Swiss Institute for Bioinformatics, for example, PSort, TargetP, ChloroP, LocTree, Predotar, LipoP, MITOPROT, PATS, PTS1, SignalP and others. The identification of subcellular localisation of the polypeptide of the invention is shown in Example 6. A predicted nuclear localisation signal (NLS) is found in the AHL19/20 polypeptide of SEQ ID NO: 2. An NLS is one or more short sequences of positively charged lysines or arginines. In particular, SEQ ID NO: 2 of the present invention is predicted to localise to the nuclear compartment of eucaryotic cells.

[0139] Furthermore, AHL19/20 polypeptides useful in the methods of the present invention (at least in their native form) typically, but not necessarily, have transcriptional regulatory activity and capacity to interact with other proteins. Therefore, AHL19/20 polypeptides with reduced transcriptional regulatory activity, without transcriptional regulatory activity, with reduced protein-protein interaction capacity, or with no protein-protein interaction capacity, may equally be useful in the methods of the present invention. DNA-binding activity and protein-protein interactions may readily be determined in vitro or in vivo using techniques well known in the art (for example in Current Protocols in Molecular Biology, Volumes 1 and 2, Ausubel et al. (1994), Current Protocols). To determine the DNA binding activity of AHL19/20 polypeptides, several assays are available, such as DNA binding gel-shift assays (or gel retardation assays; Korfhage et al. (1994) Plant C 6: 695-708), *in vitro* DNA binding assays (Schindler et al. (1993) Plant J 4(1): 137-150), or transcriptional activation of AHL19/20 polypeptides in yeast, animal and plant cells (Halbach et al. (2000) Nucleic Acid Res 28(18): 3542-3550). Specific DNA binding sequences can be determined using the random oligonucleotide selection technique (Viola & Gonzalez (May 26, 2007) Biochemistry).

[0140] In one embodiment the present invention is illustrated by transforming plants with the nucleic acid sequence represented by SEQ ID NO: 1, encoding the AHL19/20 polypeptide sequence of SEQ ID NO: 2. However, performance of the invention is not restricted to these sequences; the methods of the invention may advantageously be performed using any nucleic acid sequence encoding an AHL19/20 polypeptide as defined herein.

[0141] Examples of nucleic acid sequences encoding AHL19/20 polypeptides are given in Table A of Example 1 herein. Such nucleic acid sequences are useful in performing the methods of the invention. The polypeptide sequences given in Table A of Example 1 are example sequences of orthologues and paralogues of the AHL19/20 polypeptide represented by SEQ ID NO: 2, the terms "orthologues" and "paralogues" being as defined herein. Further orthologues and paralogues may readily be identified by performing a so-called reciprocal blast search. Typically, this involves a first BLAST involving BLASTing a query sequence (for example using any of the sequences listed in Table A of Example 1) against any sequence database, such as the publicly available NCBI database. BLASTN or TBLASTX (using standard default values) are generally used when starting from a nucleotide sequence, and BLASTP or TBLASTN (using standard default values) when starting from a protein sequence. The BLAST results may optionally be filtered. The full-length sequences of either the filtered results or non-filtered results are then BLASTed back (second BLAST) against sequences from the organism from which the query sequence is derived (where the query sequence is SEQ ID NO: 1 or SEQ ID NO: 2, the second BLAST would therefore be against *Arabidopsis thaliana* sequences). The results of the first and second BLASTs are then compared. A paralogue is identified if a high-ranking hit from the first blast is from the same species as from which

the query sequence is derived, a BLAST back then ideally results in the query sequence amongst the highest hits; an orthologue is identified if a high-ranking hit in the first BLAST is not from the same species as from which the query sequence is derived, and preferably results upon BLAST back in the query sequence being among the highest hits.

[0142] Furthermore, GRP polypeptides, as far as SEQ ID NO: 46, and its orthologues, paralogues, and homologues are concerned, typically have metal binding activity which can be measured in a metal saturation test (Scheuhammer et al., Toxicol. Appl Pharmacol. 82, 417-425, 1986) and/or may function as a redox sensor (Fabisiak et al., Methods Enzymol. 353, 268-281 (2002)).

[0143] In one embodiment the present invention is illustrated by transforming plants with the nucleic acid sequence represented by SEQ ID NO: 45, encoding the polypeptide sequence of SEQ ID NO: 46. However, performance of the invention is not restricted to these sequences; the methods of the invention may advantageously be performed using any GRP-encoding nucleic acid sequence or GRP polypeptide as defined herein.

[0144] Examples of nucleic acid sequences encoding GRP polypeptides may be found in databases known in the art. Such nucleic acid sequences are useful in performing the methods of the invention. Orthologues and paralogues, the terms "orthologues" and "paralogues" being as defined herein, may readily be identified by performing a so-called reciprocal blast search. Typically, this involves a first BLAST involving BLASTing a query sequence (for example using SEQ ID NO: 46) against any sequence database, such as the publicly available NCBI database. BLASTN or TBLASTX (using standard default values) are generally used when starting from a nucleotide sequence, and BLASTP or TBLASTN (using standard default values) when starting from a protein sequence. The BLAST results may optionally be filtered. The full-length sequences of either the filtered results or non-filtered results are then BLASTed back (second BLAST) against sequences from the organism from which the query sequence is derived (where the query sequence is SEQ ID NO: 45 or SEQ ID NO: 46, the second BLAST would therefore be against *Arabidopsis thaliana* sequences). The results of the first and second BLASTs are then compared. A paralogue is identified if a high-ranking hit from the first blast is from the same species as from which the query sequence is derived, a BLAST back then ideally results in the query sequence amongst the highest hits; an orthologue is identified if a high-ranking hit in the first BLAST is not from the same species as from which the query sequence is derived, and preferably results upon BLAST back in the query sequence being among the highest hits.

[0145] In one embodiment the present invention is illustrated by transforming plants with the nucleic acid sequence represented by SEQ ID NO: 50, encoding the polypeptide sequence of SEQ ID NO: 51. However, performance of the invention is not restricted to these sequences; the methods of the invention may advantageously be performed using any AAT-like nucleic acid or AAT-like polypeptide as defined herein.

[0146] Examples of nucleic acids useful in performing the methods of the invention include orthologues and paralogues of the AAT-like polypeptide represented by SEQ ID NO: 51, the terms "orthologues" and "paralogues" being as defined herein. Orthologues and paralogues may readily be identified by performing a so-called reciprocal blast search. Typically, this involves a first BLAST involving BLASTing a query sequence (for example SEQ ID NO: 50 or SEQ ID NO: 51) against any sequence database, such as the publicly available NCBI database. BLASTN or TBLASTX (using standard default values) are generally used when starting from a nucleotide sequence, and BLASTP or TBLASTN (using standard default values) when starting from a protein sequence. The BLAST results may optionally be filtered. The full-length sequences of either the filtered results or non-filtered results are then BLASTed back (second BLAST) against sequences from the organism from which the query sequence is derived (where the query sequence is SEQ ID NO: 50 or SEQ ID NO: 51, the second BLAST would therefore be against sequences from *Chlamydomonas*). The results of the first and second BLASTs are then compared. A paralogue is identified if a high-ranking hit from the first blast is from the same species as from which the query sequence is derived, a BLAST back then ideally results in the query sequence amongst the highest hits; an orthologue is identified if a high-ranking hit in the first BLAST is not from the same species as from which the query sequence is derived, and preferably results upon BLAST back in the query sequence being among the highest hits.

[0147] In one embodiment the present invention is illustrated by transforming plants with the nucleic acid sequence represented by SEQ ID NO: 55, encoding the polypeptide sequence of SEQ ID NO: 56. However, performance of the invention is not restricted to these sequences; the methods of the invention may advantageously be performed using any AAT-encoding nucleic acid or AAT polypeptide as defined herein.

[0148] An example of how to find nucleic acids encoding AAT polypeptides and orthologues and paralogues thereof is given in Example 1 herein. Such nucleic acids are useful in performing the methods of the invention. Orthologues and paralogues may readily be identified by performing a so-called reciprocal blast search. Typically, this involves a first BLAST involving BLASTing a query sequence (for example using SEQ ID NO: 55 or SEQ ID NO: 56) against any sequence database, such as the publicly available NCBI database. BLASTN or TBLASTX (using standard default values) are generally used when starting from a nucleotide sequence, and BLASTP or TBLASTN (using standard default values) when starting from a protein sequence. The BLAST results may optionally be filtered. The full-length sequences of either the filtered results or non-filtered results are then BLASTed back (second BLAST) against sequences from the organism from which the query sequence is derived (where the query sequence is SEQ ID NO: 55 or SEQ ID NO: 56, the second

BLAST would therefore be against rice sequences). The results of the first and second BLASTs are then compared. A paralogue is identified if a high-ranking hit from the first blast is from the same species as from which the query sequence is derived, a BLAST back then ideally results in the query sequence amongst the highest hits; an orthologue is identified if a high-ranking hit in the first BLAST is not from the same species as from which the query sequence is derived, and preferably results upon BLAST back in the query sequence being among the highest hits.

[0149] High-ranking hits are those having a low E-value. The lower the E-value, the more significant the score (or in other words the lower the chance that the hit was found by chance). Computation of the E-value is well known in the art. In addition to E-values, comparisons are also scored by percentage identity. Percentage identity refers to the number of identical nucleotides (or amino acids) between the two compared nucleic acid (or polypeptide) sequences over a particular length. In the case of large families, ClustalW may be used, followed by a neighbour joining tree, to help visualize clustering of related genes and to identify orthologues and paralogues. Any sequence clustering within the group comprising SEQ ID NO: 2 (AHL19 polypeptide; encircled in Figures 1 and 2) would be considered to fall within the aforementioned definition of an AHL19/20 polypeptide, and would be considered suitable for use in the methods of the invention.

[0150] Nucleic acid variants may also be useful in practising the methods of the invention. Examples of such variants include nucleic acid sequences encoding homologues and derivatives of any one of the polypeptide sequences given in Table A of Example 1, the terms "homologue" and "derivative" being as defined herein. Also useful in the methods of the invention are nucleic acid sequences encoding homologues and derivatives of orthologues or paralogues of any one of the polypeptide sequences given in Table A of Example 1. Homologues and derivatives useful in the methods of the present invention have substantially the same biological and functional activity as the unmodified protein from which they are derived.

[0151] Further nucleic acid variants useful in practising the methods of the invention include portions of nucleic acid sequences encoding AHL19/20 polypeptides, nucleic acid sequences hybridising to nucleic acid sequences encoding AHL19/20 polypeptides, splice variants of nucleic acid sequences encoding AHL19/20 polypeptides, allelic variants of nucleic acid sequences encoding AHL19/20 polypeptides and variants of nucleic acid sequences encoding AHL19/20 polypeptides obtained by gene shuffling. The terms hybridising sequence, splice variant, allelic variant and gene shuffling are as described herein.

[0152] Nucleic acid sequences encoding AHL19/20 polypeptides need not be full-length nucleic acid sequences, since performance of the methods of the invention does not rely on the use of full-length nucleic acid sequences. According to the present invention, there is provided a method for increasing seed yield-related traits, in plants, comprising introducing and expressing in a plant a portion of any one of the nucleic acid sequences given in Table A of Example 1, or a portion of a nucleic acid sequence encoding an orthologue, paralogue or homologue of any of the polypeptide sequences given in Table A of Example 1.

[0153] Nucleic acid sequence variants encoding homologues and derivatives of SEQ ID NO: 46 may also be useful in practising the methods of the invention, the terms "homologue" and "derivative" being as defined herein. Also useful in the methods of the invention are nucleic acid sequences encoding homologues and derivatives of orthologues or paralogues of SEQ ID NO: 46. Homologues and derivatives useful in the methods of the present invention have substantially the same biological and functional activity as the unmodified protein from which they are derived.

[0154] Further nucleic acid sequence variants useful in practising the methods of the invention include portions of nucleic acid sequences encoding GRP polypeptides, nucleic acid sequences hybridising to nucleic acid sequences encoding GRP polypeptides, splice variants of nucleic acid sequences encoding GRP polypeptides, allelic variants of nucleic acid sequences encoding GRP polypeptides and variants of nucleic acid sequences encoding GRP polypeptides obtained by gene shuffling. The terms hybridising sequence, splice variant, allelic variant and gene shuffling are as described herein.

[0155] Nucleic acid sequences encoding GRP polypeptides need not be full-length nucleic acid sequences, since performance of the methods of the invention does not rely on the use of full-length nucleic acid sequences. According to the present invention, there is provided a method for enhancing yield-related traits in plants grown under abiotic stress conditions, comprising introducing and expressing in a plant a portion of SEQ ID NO: 45, or a portion of a nucleic acid sequence encoding an orthologue, paralogue, or homologue of SEQ ID NO: 46.

[0156] Nucleic acid variants may also be useful in practising the methods of the invention. Examples of such variants include nucleic acids encoding homologues and derivatives of SEQ ID NO: 51, the terms "homologue" and "derivative" being as defined herein. Also useful in the methods of the invention are nucleic acids encoding homologues and derivatives of orthologues or paralogues of the AAT-like polypeptide represented by SEQ ID NO: 51 or AAT polypeptide represented by SEQ ID NO: 56. Homologues and derivatives useful in the methods of the present invention have substantially the same biological and functional activity as the unmodified protein from which they are derived.

[0157] Further nucleic acid variants useful in practising the methods of the invention include portions of nucleic acids encoding AAT-like polypeptides or AAT polypeptide, nucleic acids hybridising to nucleic acids encoding AAT-like polypeptides or AAT polypeptide, splice variants of nucleic acids encoding AAT-like polypeptides or AAT polypeptide, allelic

variants of nucleic acids encoding AAT-like polypeptides or AAT polypeptide and variants of nucleic acids encoding AAT-like polypeptides or AAT polypeptide obtained by gene shuffling. The terms hybridising sequence, splice variant, allelic variant and gene shuffling are as described herein.

[0158] Nucleic acids encoding AAT-like polypeptides or AAT polypeptide need not be full-length nucleic acids, since performance of the methods of the invention does not rely on the use of full-length nucleic acid sequences. According to the present invention, there is provided a method for enhancing yield-related traits in plants, comprising introducing and expressing in a plant a portion of SEQ ID NO: 50 or SEQ ID NO: 55 or a portion of a nucleic acid encoding an orthologue, paralogue or homologue of SEQ ID NO: 51 or SEQ ID NO: 56.

[0159] A portion of a nucleic acid sequence may be prepared, for example, by making one or more deletions to the nucleic acid sequence. The portions may be used in isolated form or they may be fused to other coding (or non-coding) sequences in order to, for example, produce a protein that combines several activities. When fused to other coding sequences, the resultant polypeptide produced upon translation may be bigger than that predicted for the protein portion.

[0160] Portions useful in the methods of the invention, encode an AHL19/20 polypeptide as defined herein, and have substantially the same biological activity as the polypeptide sequences given in Table A of Example 1. Preferably, the portion is a portion of any one of the nucleic acid sequences given in Table A of Example 1, or is a portion of a nucleic acid sequence encoding an orthologue or paralogue of any one of the polypeptide sequences given in Table A of Example 1. Preferably the portion is, in increasing order of preference at least 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 940 consecutive nucleotides in length, the consecutive nucleotides being of any one of the nucleic acid sequences given in Table A of Example 1, or of a nucleic acid sequence encoding an orthologue or paralogue of any one of the polypeptide sequences given in Table A of Example 1. Preferably, the portion is a portion of a nucleic acid sequence encoding a polypeptide sequence which when used in the construction of an AHL phylogenetic tree, such as the one depicted in Figure 1 or in Figure 2, clusters with the group of AHL19/20 polypeptides comprising the polypeptide sequence represented by SEQ ID NO: 2 rather than with any other AHL group. Most preferably the portion is a portion of the nucleic acid sequence of SEQ ID NO: 1.

[0161] Portions useful in the methods of the invention, encode a GRP polypeptide as defined herein, and have substantially the same biological activity as the polypeptide sequences given in SEQ ID NO: 46. Preferably, the portion is a portion of the nucleic acid sequence given in SEQ ID NO: 45, or is a portion of a nucleic acid sequence encoding an orthologue or paralogue of the polypeptide sequence given in SEQ ID NO: 46. Preferably the portion is at least 50, 75, 100, 125, 150, 175, 200, 210, 220, 230, 240, or more consecutive nucleotides in length, the consecutive nucleotides being of SEQ ID NO: 45, or of a nucleic acid sequence encoding an orthologue or paralogue of SEQ ID NO: 46. Most preferably the portion is a portion of the nucleic acid sequence of SEQ ID NO: 45.

[0162] Portions useful in the methods of the invention, encode an AAT-like polypeptide as defined herein, and have substantially the same biological activity as the amino acid sequence of SEQ ID NO: 51. Preferably the portion is at least 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, 1400, 1450, 1500, 1550 consecutive nucleotides in length, the consecutive nucleotides being of SEQ ID NO: 50 or of a nucleic acid sequence encoding an orthologue or paralogue of SEQ ID NO: 51.

[0163] Preferably, the portion encodes a fragment of an amino acid sequence which, when used in the construction of a phylogenetic tree containing AAT sequences, clusters with the group of AAT-like polypeptides comprising SEQ ID NO: 51 rather than with any other group of AATs or AAT-like sequences.

[0164] Portions useful in the methods of the invention, encode an AAT polypeptide as defined herein, and have substantially the same biological activity as the amino acid sequence of SEQ ID NO: 56. Preferably the portion is at least 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, 1400, 1450 consecutive nucleotides in length, the consecutive nucleotides being of SEQ ID NO: 55 or of a nucleic acid sequences encoding an orthologue or paralogue of SEQ ID NO: 56.

[0165] Preferably, the portion encodes a fragment of an amino acid sequence which, when used in the construction of a phylogenetic tree containing AAT sequences, clusters with the group of AAT polypeptides comprising SEQ ID NO: 56 rather than with any other group of AAT sequences.

[0166] Another nucleic acid sequence variant useful in the methods of the invention is a nucleic acid sequence capable of hybridising, under reduced stringency conditions, preferably under stringent conditions, with a nucleic acid sequence encoding a yield increasing polypeptide selected from the group consisting of: an AT-hook motif nuclear localized 19/20 (AHL19/20), a GRP (Growth Regulating Protein wherein said GRP polypeptide is a metallothionein 2a (MT2a) polypeptide)" an alanine aminotransferase (AAT)-like polypeptide and an alanine aminotransferase (AAT) polypeptide as defined herein, or with a portion as defined herein.

[0167] According to the present invention, in one embodiment there is provided a method for increasing seed yield-related traits in plants, comprising introducing and expressing in a plant a nucleic acid sequence capable of hybridizing to any one of the nucleic acid sequences given in Table A of Example 1, or comprising introducing and expressing in a plant a nucleic acid sequence capable of hybridising to a nucleic acid sequence encoding an orthologue, paralogue or homologue of any of the nucleic acid sequences given in Table A of Example 1.

[0168] Hybridising sequences useful in the methods of the invention encode an AHL19/20 polypeptide as defined herein, and have substantially the same biological activity as the polypeptide sequences given in Table A of Example 1. Preferably, the hybridising sequence is capable of hybridising to any one of the nucleic acid sequences given in Table A of Example 1, or to a portion of any of these sequences, a portion being as defined above, or wherein the hybridising sequence is capable of hybridising to a nucleic acid sequence encoding an orthologue or paralogue of any one of the polypeptide sequences given in Table A of Example 1. Preferably, the hybridising sequence is capable of hybridising to a nucleic acid sequence encoding a polypeptide sequence which when used in the construction of an AHL phylogenetic tree, such as the one depicted in Figure 1 or in Figure 2, clusters with the group of AHL19/20 polypeptides comprising the polypeptide sequence represented by SEQ ID NO: 2 rather than with any other AHL group. Most preferably, the hybridising sequence is capable of hybridising to a nucleic acid sequence as represented by SEQ ID NO: 1 or to a portion thereof.

[0169] According to the present invention, in one embodiment there is provided a method for enhancing yield-related traits in plants grown under abiotic stress conditions, comprising introducing and expressing in a plant a nucleic acid sequence capable of hybridizing to SEQ ID NO: 45, or comprising introducing and expressing in a plant a nucleic acid sequence capable of hybridising to a nucleic acid sequence encoding an orthologue, paralogue, or homologue of SEQ ID NO: 46.

[0170] Hybridising sequences useful in the methods of the invention encode a GRP polypeptide as defined herein, having substantially the same biological activity as the polypeptide sequence given in SEQ ID NO: 46. Preferably, the hybridising sequence is capable of hybridising to SEQ ID NO: 45, or to a portion of this sequence, a portion being as defined above, or the hybridising sequence is capable of hybridising to a nucleic acid sequence encoding an orthologue or paralogue of SEQ ID NO: 46, or to a portion thereof.

[0171] According to the present invention, in one embodiment there is provided a method for enhancing yield-related traits in plants, comprising introducing and expressing in a plant a nucleic acid capable of hybridizing to SEQ ID NO: 50 or capable of hybridising to a nucleic acid encoding an orthologue, paralogue or homologue of SEQ ID NO: 51.

[0172] Hybridising sequences useful in the methods of the invention encode an AAT-like polypeptide as defined herein, having substantially the same biological activity as the amino acid sequence of SEQ ID NO: 51. Preferably, the hybridising sequence is capable of hybridising to a nucleic acid encoding an orthologue, paralogue or homologue of SEQ ID NO: 51, or to a portion of such nucleic acid, a portion being as defined above. Most preferably, the hybridising sequence is capable of hybridising to a nucleic acid as represented by SEQ ID NO: 50 or to a portion thereof.

[0173] According to the present invention, there is provided a method for enhancing yield-related traits in plants, comprising introducing and expressing in a plant a nucleic acid capable of hybridizing to SEQ ID NO: 55 or capable of hybridising to a nucleic acid encoding an orthologue, paralogue or homologue of SEQ ID NO: 56.

[0174] Hybridising sequences useful in the methods of the invention encode an AAT polypeptide as defined herein, having substantially the same biological activity as the amino acid sequence of SEQ ID NO: 56. Preferably, the hybridising sequence is capable of hybridising to a nucleic acid encoding an orthologue, paralogue or homologue of SEQ ID NO: 56, or to a portion of such nucleic acid, a portion being as defined above. Most preferably, the hybridising sequence is capable of hybridising to a nucleic acid as represented by SEQ ID NO: 55 or to a portion thereof.

[0175] Preferably, the hybridising sequence encodes a polypeptide with an amino acid sequence which, when full-length and when used in the construction of a phylogenetic tree containing AAT sequences, clusters with the group of AAT-like polypeptides comprising SEQ ID NO: 51 or clusters with the group of AAT polypeptides comprising SEQ ID NO: 56 rather than with any other group of AATs or AAT-like sequences.

[0176] Another nucleic acid sequence variant useful in the methods of the invention is a splice variant encoding a yield increasing polypeptide selected from the group consisting of: an AT-hook motif nuclear localized 19/20 (AHL19/20), a GRP (Growth Regulating Protein, wherein said GRP polypeptide is a metallothionein 2a (MT2a) polypeptide), , an alanine aminotransferase (AAT)-like polypeptide and an alanine aminotransferase (AAT) polypeptide as defined hereinabove, a splice variant being as defined herein.

[0177] According to the present invention, there is provided a method for increasing seed yield-related traits, comprising introducing and expressing in a plant a splice variant of any one of the nucleic acid sequences given in Table A of Example 1, or a splice variant of a nucleic acid sequence encoding an orthologue, paralogue or homologue of any of the polypeptide sequences given in Table A of Example 1.

[0178] In one embodiment splice variants are splice variants of a nucleic acid sequence represented by SEQ ID NO: 1, or a splice variant of a nucleic acid sequence encoding an orthologue or paralogue of SEQ ID NO: 2. Preferably, the splice variant is a splice variant of a nucleic acid sequence encoding a polypeptide sequence which when used in the construction of a AHL phylogenetic tree, such as the one depicted in Figure 1 or in Figure 2, clusters with the group of AHL19/20 polypeptides comprising the polypeptide sequence represented by SEQ ID NO: 2 rather than with any other AHL group.

[0179] In one embodiment according to the present invention, there is provided a method for enhancing yield-related traits in plants grown under abiotic stress conditions relative to control plants, comprising introducing and expressing in

a plant a splice variant of SEQ ID NO: 45, or a splice variant of a nucleic acid sequence encoding an orthologue, paralogue, or homologue of SEQ ID NO: 46.

[0180] In one embodiment according to the present invention, there is provided a method for enhancing yield-related traits in plants, comprising introducing and expressing in a plant a splice variant of SEQ ID NO: 50 or SEQ ID NO: 55, or a splice variant of a nucleic acid encoding an orthologue, paralogue or homologue of the amino acid sequence of SEQ ID NO: 51 or SEQ ID NO: 56.

[0181] Preferably, the amino acid sequence encoded by the splice variant, when used in the construction of a phylogenetic tree containing AAT sequences, clusters with the group of AAT-like polypeptides comprising SEQ ID NO: 51 or clusters with the group of AAT polypeptides comprising SEQ ID NO: 56 rather than with any other group of AATs or AAT-like sequences.

[0182] Another nucleic acid sequence variant useful in performing the methods of the invention is an allelic variant of a nucleic acid sequence encoding a yield increasing polypeptide selected from the group consisting of: an AT-hook motif nuclear localized 19/20 (AHL19/20), GRP (Growth Regulating Protein, wherein said GRP polypeptide is a metallothionein 2a (MT2a) polypeptide), , an alanine aminotransferase (AAT)-like polypeptide and an alanine aminotransferase (AAT) polypeptide as defined hereinabove, an allelic variant being as defined herein.

[0183] In one embodiment according to the present invention, there is provided a method for increasing seed yield-related traits, comprising introducing and expressing in a plant an allelic variant of any one of the nucleic acid sequences given in Table A of Example 1, or comprising introducing and expressing in a plant an allelic variant of a nucleic acid sequence encoding an orthologue, paralogue or homologue of any of the polypeptide sequences given in Table A of Example 1.

[0184] The allelic variants useful in the methods of the present invention have substantially the same biological activity as the AHL19/20 polypeptide of SEQ ID NO: 2 and any of the polypeptide sequences depicted in Table A of Example 1. Allelic variants exist in nature, and encompassed within the methods of the present invention is the use of these natural alleles. Preferably, the allelic variant is an allelic variant of SEQ ID NO: 1 or an allelic variant of a nucleic acid sequence encoding an orthologue or paralogue of SEQ ID NO: 2. Preferably, the allelic variant is an allelic variant of a polypeptide sequence which when used in the construction of a AHL phylogenetic tree, such as the one depicted in Figure 1 or in Figure 2, clusters with the AHL19/20 polypeptides comprising the polypeptide sequence represented by SEQ ID NO: 2 rather than with any other AHL group.

[0185] In one embodiment according to the present invention, there is provided a method for enhancing yield-related traits in plants grown under abiotic stress conditions, comprising introducing and expressing in a plant an allelic variant of SEQ ID NO: 45, or comprising introducing and expressing in a plant an allelic variant of a nucleic acid sequence encoding an orthologue, paralogue, or homologue of the polypeptide sequence represented by SEQ ID NO: 46.

[0186] The allelic variants useful in the methods of the present invention have substantially the same biological activity as the GRP polypeptide of SEQ ID NO: 46. Allelic variants exist in nature, and encompassed within the methods of the present invention is the use of these natural alleles.

[0187] In one embodiment according to the present invention, there is provided a method for enhancing yield-related traits in plants, comprising introducing and expressing in a plant an allelic variant of SEQ ID NO: 50 or SEQ ID NO: 55, or an allelic variant of a nucleic acid encoding an orthologue, paralogue or homologue of the amino acid sequence of SEQ ID NO: 51 or SEQ ID NO: 56.

[0188] The allelic variants useful in the methods of the present invention have substantially the same biological activity as the AAT-like polypeptide of SEQ ID NO: 51 or as the AAT polypeptide of SEQ ID NO: 56 respectively. Allelic variants exist in nature, and encompassed within the methods of the present invention is the use of these natural alleles. Preferably, the amino acid sequence encoded by the allelic variant, when used in the construction of a phylogenetic tree containing AAT sequences, clusters with the group of AAT-like polypeptides comprising SEQ ID NO: 51 or clusters with the group of AAT polypeptides comprising SEQ ID NO: 56 rather than with any other group of AATs or AAT-like sequences.

[0189] Gene shuffling or directed evolution may also be used to generate variants of nucleic acid sequences encoding yield increasing polypeptides selected from the group consisting of: an AT-hook motif nuclear localized 19/20 (AHL19/20), GRP (Growth Regulating Protein, wherein said GRP polypeptide is a metallothionein 2a (MT2a) polypeptide), , an alanine aminotransferase (AAT)-like polypeptide and an alanine aminotransferase (AAT) polypeptide as defined above, the term "gene shuffling" being as defined herein.

[0190] In one embodiment according to the present invention, there is provided a method for increasing seed yield-related traits, comprising introducing and expressing in a plant a variant of any one of the nucleic acid sequences given in Table A of Example 1, or comprising introducing and expressing in a plant a variant of a nucleic acid sequence encoding an orthologue, paralogue or homologue of any of the polypeptide sequences given in Table A of Example 1, which variant nucleic acid sequence is obtained by gene shuffling.

[0191] Preferably, the variant nucleic acid sequence obtained by gene shuffling encodes a polypeptide sequence which when used in the construction of a AHL phylogenetic tree, such as the one depicted in Figure 1 and in Figure 2, clusters with the group of AHL19/20 polypeptides comprising the polypeptide sequence represented by SEQ ID NO: 2

rather than with any other AHL group.

[0192] In one embodiment according to the present invention, there is provided a method for enhancing yield-related traits in plants grown under abiotic stress conditions, comprising introducing and expressing in a plant a variant nucleic acid sequence of SEQ ID NO: 45, or comprising introducing and expressing in a plant a variant of a nucleic acid sequence encoding an orthologue, paralogue, or homologue of SEQ ID NO: 46, which variant nucleic acid sequence is obtained by gene shuffling.

[0193] In one embodiment according to the present invention, there is provided a method for enhancing yield-related traits in plants, comprising introducing and expressing in a plant a variant of SEQ ID NO: 50 or of SEQ ID NO: 55, or a variant of a nucleic acid encoding an orthologue, paralogue or homologue of SEQ ID NO: 51 of SEQ ID NO: 56, which variant nucleic acid is obtained by gene shuffling.

[0194] Preferably, the amino acid sequence encoded by the variant nucleic acid obtained by gene shuffling, when used in the construction of a phylogenetic tree containing AAT sequences, clusters with the group of AAT-like polypeptides comprising SEQ ID NO: 51 or clusters with the group of AAT polypeptides comprising SEQ ID NO: 56 rather than with any other group of AATs or AAT-like sequences.

[0195] Furthermore, nucleic acid sequence variants may also be obtained by site-directed mutagenesis. Several methods are available to achieve site-directed mutagenesis, the most common being PCR based methods (Current Protocols in Molecular Biology. Wiley Eds.).

[0196] Nucleic acid sequences encoding AHL19/20 polypeptides may be derived from any natural or artificial source. The nucleic acid sequence may be modified from its native form in composition and/or genomic environment through deliberate human manipulation. Preferably the nucleic acid sequence encoding an AHL19/20 polypeptide is from a plant, further preferably from a dicotyledonous plant, more preferably from the family Brassicaceae, most preferably the nucleic acid sequence is from *Arabidopsis thaliana*.

[0197] Nucleic acid sequences encoding GRP polypeptides may be derived from any natural or artificial source. The nucleic acid sequence may be modified from its native form in composition and/or genomic environment through deliberate human manipulation. Preferably the GRP polypeptide-encoding nucleic acid sequence is from a plant. In the case of SEQ ID NO: 45, the GRP polypeptide encoding nucleic acid sequence is preferably from a dicotyledonous plant, more preferably from the family Brassicaceae, most preferably the nucleic acid sequence is from *Arabidopsis thaliana*.

[0198] Performance of the methods of the invention gives plants having increased seed yield-related traits relative to control plants. The terms "yield" and "seed yield" are described in more detail in the "definitions" section herein.

[0199] Performance of the methods of the invention gives plants grown under abiotic stress conditions having enhanced yield-related traits relative to control plants. In particular, performance of the methods of the invention gives plants grown under abiotic stress conditions having increased early vigour and increased yield, especially increased biomass and increased seed yield, relative to control plants. The terms "yield" and "seed yield" are described in more detail in the "definitions" section herein.

[0200] Reference herein to enhanced yield-related traits is taken to mean an increase in early vigour and/or in biomass (weight) of one or more parts of a plant, which may include aboveground (harvestable) parts and/or (harvestable) parts below ground. In particular, such harvestable parts are biomass and/or seeds, and performance of the methods of the invention results in plants grown under abiotic stress conditions having increased early vigour, biomass and/or seed yield relative to the early vigour, biomass or seed yield of control plants grown under comparable conditions.

[0201] Nucleic acids encoding AAT-like polypeptides may be derived from any natural or artificial source. The nucleic acid may be modified from its native form in composition and/or genomic environment through deliberate human manipulation. Preferably the AAT-like nucleic acid is of algal origin, preferably from the genus *Chlamydomonas*, further preferably from the species *Chlamydomonas reinhardtii*.

[0202] Performance of the methods of the invention gives plants having enhanced yield-related traits. In particular performance of the methods of the invention gives plants having increased yield, especially increased seed yield relative to control plants. The terms "yield" and "seed yield" are described in more detail in the "definitions" section herein.

[0203] Reference herein to enhanced yield-related traits is taken to mean an increase in biomass (weight) of one or more parts of a plant, which may include aboveground (harvestable) parts and/or (harvestable) parts below ground. In particular, such harvestable parts are seeds.

[0204] Nucleic acids encoding AAT polypeptides may be derived from any natural or artificial source. The nucleic acid may be modified from its native form in composition and/or genomic environment through deliberate human manipulation. Preferably the POI polypeptide-encoding nucleic acid is from a plant, further preferably from a monocotyledonous plant, more preferably from the family Poaceae, most preferably the nucleic acid is from *Oryza sativa*.

[0205] Reference herein to enhanced yield-related traits is taken to mean an increase in biomass (weight) of one or more parts of a plant, which may include aboveground (harvestable) parts and/or (harvestable) parts below ground. In particular, such harvestable parts are seeds, and performance of the methods of the invention results in plants having increased seed yield relative to the seed yield of control plants.

[0206] Taking corn as an example, a yield increase may be manifested as one or more of the following: increase in

the number of plants established per hectare or acre, an increase in the number of ears per plant, an increase in the number of rows, number of kernels per row, kernel weight, thousand kernel weight, ear length/diameter, increase in the seed filling rate (which is the number of filled seeds divided by the total number of seeds and multiplied by 100), among others. Taking rice as an example, a yield increase may manifest itself as an increase in one or more of the following:

number of plants per hectare or acre, number of panicles per plant, number of spikelets per panicle, number of flowers (florets) per panicle (which is expressed as a ratio of the number of filled seeds over the number of primary panicles), increase in the seed filling rate (which is the number of filled seeds divided by the total number of seeds and multiplied by 100), increase in thousand kernel weight, among others.

[0207] In one embodiment the present invention provides a method for increasing seed yield-related traits of plants relative to control plants, which method comprises increasing expression in a plant of a nucleic acid sequence encoding an AHL19/20 polypeptide as defined herein.

[0208] Since the transgenic plants according to the present invention have increased seed yield-related traits, it is likely that these plants exhibit an increased growth rate (during at least part of their life cycle), relative to the growth rate of control plants at a corresponding stage in their life cycle.

[0209] In one embodiment the present invention provides a method for enhancing plant yield-related traits under abiotic stress growth conditions, especially biomass and/or seed yield of plants, relative to control plants grown under comparable conditions, which method comprises increasing expression in a plant of a nucleic acid sequence encoding a GRP polypeptide as defined herein.

[0210] Since the transgenic plants according to the present invention grown under abiotic stress conditions have enhanced yield-related traits, it is likely that these plants exhibit an increased growth rate (during at least part of their life cycle), relative to the growth rate of control plants at a corresponding stage in their life cycle, and under comparably growth conditions.

[0211] In one embodiment the present invention provides a method for increasing yield, especially seed yield of plants, relative to control plants, which method comprises modulating expression, preferably increasing expression, in a plant of a nucleic acid encoding an AAT-like polypeptide or AAT polypeptide as defined herein.

[0212] Since the transgenic plants according to the present invention have increased yield, it is likely that these plants exhibit an increased growth rate (during at least part of their life cycle), relative to the growth rate of control plants at a corresponding stage in their life cycle.

[0213] The increased growth rate may be specific to one or more parts of a plant (including seeds), or may be throughout substantially the whole plant. Plants having an increased growth rate may have a shorter life cycle. The life cycle of a plant may be taken to mean the time needed to grow from a dry mature seed up to the stage where the plant has produced dry mature seeds, similar to the starting material. This life cycle may be influenced by factors such as early vigour, growth rate, greenness index, flowering time and speed of seed maturation. The increase in growth rate may take place at one or more stages in the life cycle of a plant or during substantially the whole plant life cycle. Increased growth rate during the early stages in the life cycle of a plant may reflect increased (early) vigour. The increase in growth rate may alter the harvest cycle of a plant allowing plants to be sown later and/or harvested sooner than would otherwise be possible (a similar effect may be obtained with earlier flowering time; delayed flowering is usually not a desired trait in crops). If the growth rate is sufficiently increased, it may allow for the further sowing of seeds of the same plant species (for example sowing and harvesting of rice plants followed by sowing and harvesting of further rice plants all within one conventional growing period). Similarly, if the growth rate is sufficiently increased, it may allow for the further sowing of seeds of different plants species (for example the sowing and harvesting of corn plants followed by, for example, the sowing and optional harvesting of soybean, potato or any other suitable plant). Harvesting additional times from the same rootstock in the case of some crop plants may also be possible. Altering the harvest cycle of a plant may lead to an increase in annual biomass production per acre (due to an increase in the number of times (say in a year) that any particular plant may be grown and harvested). An increase in growth rate may also allow for the cultivation of transgenic plants in a wider geographical area than their wild-type counterparts, since the territorial limitations for growing a crop are often determined by adverse environmental conditions either at the time of planting (early season) or at the time of harvesting (late season). Such adverse conditions may be avoided if the harvest cycle is shortened. The growth rate may be determined by deriving various parameters from growth curves, such parameters may be: T-Mid (the time taken for plants to reach 50% of their maximal size) and T-90 (time taken for plants to reach 90% of their maximal size), amongst others. The growth rate defined herein is not taken to mean delayed flowering.

[0214] According to one embodiment of the present invention, performance of the methods of the invention gives plants having an increased growth rate relative to control plants. Therefore, according to this embodiment of the present invention, there is provided a method for increasing the growth rate of plants, which method comprises increasing expression in a plant of a nucleic acid sequence encoding an AHL19/20 polypeptide as defined herein.

[0215] According to an embodiment of the present invention, performance of the methods of the invention gives plants grown under abiotic stress conditions having an increased growth rate, relative to control plants. Therefore, according to this embodiment of the present invention, there is provided a method for increasing the growth rate of plants grown

under abiotic stress conditions, which method comprises increasing expression in a plant of a nucleic acid sequence encoding a GRP polypeptide as defined herein.

[0216] According to an embodiment of the present invention, performance of the methods of the invention gives plants having an increased growth rate relative to control plants. Therefore, according to this embodiment of the present invention, there is provided a method for increasing the growth rate of plants, which method comprises modulating expression, preferably increasing expression, in above ground plant parts of a nucleic acid encoding an AAT-like polypeptide or AAT polypeptide as defined herein.

[0217] Increased seed yield-related traits occur whether the plant is under non-stress conditions or whether the plant is exposed to various stresses compared to control plants grown under comparable conditions. An enhancement of yield-related traits (an increase in seed yield and/or growth rate) occurs whether the plant is under non-stress conditions or whether the plant is exposed to various stresses compared to control plants.

In a particularly preferred embodiment, the methods of the present invention are performed under non-stress conditions. However, an increase in yield and/or growth rate occurs whether the plant is under non-stress conditions or whether the plant is exposed to various stresses compared to control plants.

Plants typically respond to exposure to stress by growing more slowly. In conditions of severe stress, the plant may even stop growing altogether. Mild stress on the other hand is defined herein as being any stress to which a plant is exposed which does not result in the plant ceasing to grow altogether without the capacity to resume growth. Mild stress in the sense of the invention leads to a reduction in the growth of the stressed plants of less than 40%, 35% or 30%, preferably less than 25%, 20% or 15%, more preferably less than 14%, 13%, 12%, 11% or 10% or less in comparison to the control plant under non-stress conditions. Due to advances in agricultural practices (irrigation, fertilization, pesticide treatments) severe stresses are not often encountered in cultivated crop plants. As a consequence, the compromised growth induced by mild stress is often an undesirable feature for agriculture. Mild stresses are the everyday biotic and/or abiotic (environmental) stresses to which a plant is exposed. Abiotic stresses may be due to drought or excess water, anaerobic stress, salt stress, chemical toxicity, oxidative stress and hot, cold or freezing temperatures. The abiotic stress may be an osmotic stress caused by a water stress (particularly due to drought), salt stress, oxidative stress or an ionic stress. Biotic stresses are typically those stresses caused by pathogens, such as bacteria, viruses, fungi, nematodes, and insects. The term "non-stress" conditions as used herein are those environmental conditions that allow optimal growth of plants. Persons skilled in the art are aware of normal soil conditions and climatic conditions for a given location.

[0218] As reported in Wang et al. (Planta (2003) 218: 1-14), abiotic stress leads to a series of morphological, physiological, biochemical and molecular changes that adversely affect plant growth and productivity. Drought, salinity, extreme temperatures and oxidative stress are known to be interconnected and may induce growth and cellular damage through similar mechanisms. Rabbani et al. (Plant Physiol (2003) 133: 1755-1767) describes a particularly high degree of "cross talk" between drought stress and high-salinity stress. For example, drought and/or salinisation are manifested primarily as osmotic stress, resulting in the disruption of homeostasis and ion distribution in the cell. Oxidative stress, which frequently accompanies high or low temperature, salinity or drought stress, may cause denaturing of functional and structural proteins. As a consequence, these diverse environmental stresses often activate similar cell signalling pathways and cellular responses, such as the production of stress proteins, up-regulation of anti-oxidants, accumulation of compatible solutes and growth arrest. The term "non-stress" conditions as used herein are those environmental conditions that allow optimal growth of plants. Persons skilled in the art are aware of normal soil conditions and climatic conditions for a given location.

[0219] In one embodiment performance of the methods of the invention gives plants grown under non-stress conditions or under mild stress conditions having increased seed yield-related traits, relative to control plants grown under comparable conditions. Therefore, according to one embodiment of the present invention, there is provided a method for increasing seed yield-related traits in plants grown under non-stress conditions or under mild stress conditions, which method comprises increasing expression in a plant of a nucleic acid sequence encoding an AHL19/20 polypeptide.

[0220] In one embodiment performance of the methods of the invention gives plants grown under mild stress conditions having enhanced yield-related traits, relative to control plants grown under comparable conditions. Therefore, according to one embodiment of the present invention, there is provided a method for enhancing yield-related traits in plants grown under mild stress conditions, which method comprises increasing expression in a plant of a nucleic acid sequence encoding a GRP polypeptide.

[0221] Performance of the methods according to the present invention results in plants grown under abiotic stress conditions having increased yield-related traits relative to control plants grown under comparable stress conditions. As reported in Wang et al. (Planta (2003) 218: 1-14), abiotic stress leads to a series of morphological, physiological, biochemical and molecular changes that adversely affect plant growth and productivity. Drought, salinity, extreme temperatures and oxidative stress are known to be interconnected and may induce growth and cellular damage through similar mechanisms. Rabbani et al. (Plant Physiol (2003) 133: 1755-1767) describes a particularly high degree of "cross talk" between drought stress and high-salinity stress. For example, drought and/or salinisation are manifested primarily as osmotic stress, resulting in the disruption of homeostasis and ion distribution in the cell. Oxidative stress, which

frequently accompanies high or low temperature, salinity or drought stress, may cause denaturing of functional and structural proteins. As a consequence, these diverse environmental stresses often activate similar cell signalling pathways and cellular responses, such as the production of stress proteins, up-regulation of anti-oxidants, accumulation of compatible solutes and growth arrest. Since diverse environmental stresses activate similar pathways, the exemplification of the present invention with drought stress should not be seen as a limitation to drought stress, but more as a screen to indicate the involvement of AHL19/20 polypeptides as defined above, in increasing yield-related traits relative to control plants grown in comparable stress conditions, in abiotic stresses in general.

[0222] Since diverse environmental stresses activate similar pathways, the exemplification of the present invention with drought stress and salt stress should not be seen as a limitation to drought stress or salt stress, but more as a screen to indicate the involvement of GRP polypeptides as defined above, in enhancing yield-related traits relative to control plants grown in comparable stress conditions, in abiotic stresses in general.

[0223] The term "abiotic stress" as defined herein is taken to mean any one or more of: water stress (due to drought or excess water), anaerobic stress, salt stress, temperature stress (due to hot, cold or freezing temperatures), chemical toxicity stress and oxidative stress. According to one aspect of the invention, the abiotic stress is an osmotic stress, selected from water stress, salt stress, oxidative stress and ionic stress. Preferably, the water stress is drought stress. The term salt stress is not restricted to common salt (NaCl), but may be any stress caused by one or more of: NaCl, KCl, LiCl, MgCl₂, CaCl₂, amongst others.

[0224] Preferably, the abiotic stress is drought stress. Alternatively, the abiotic stress is salt stress.

[0225] In one embodiment performance of the methods of the invention gives plants having increased seed yield-related traits, under abiotic stress conditions relative to control plants grown in comparable stress conditions. Therefore, according to the present invention, there is provided a method for increasing seed yield-related traits, in plants grown under abiotic stress conditions, which method comprises increasing expression in a plant of a nucleic acid sequence encoding an AHL19/20 polypeptide. According to one aspect of the invention, the abiotic stress is an osmotic stress, selected from one or more of the following: water stress, salt stress, oxidative stress and ionic stress.

[0226] In one embodiment performance of the methods of the invention gives plants grown under abiotic stress conditions having enhanced yield-related traits relative to control plants grown in comparable stress conditions. Therefore, according to the present invention, there is provided a method for enhancing yield-related traits in plants grown under abiotic stress conditions, which method comprises increasing expression in a plant of a nucleic acid sequence encoding a GRP polypeptide. According to one aspect of the invention, the abiotic stress is an osmotic stress, selected from one or more of the following: water stress, salt stress, oxidative stress and ionic stress. Preferably, the abiotic stress is drought stress. Alternatively or additionally, the abiotic stress is salt stress.

[0227] Another example of abiotic environmental stress is the reduced availability of one or more nutrients that need to be assimilated by the plants for growth and development. Because of the strong influence of nutrition utilization efficiency on plant yield and product quality, a huge amount of fertilizer is poured onto fields to optimize plant growth and quality. Productivity of plants ordinarily is limited by three primary nutrients, phosphorous, potassium and nitrogen, which is usually the rate-limiting element in plant growth of these three. Therefore the major nutritional element required for plant growth is nitrogen (N). It is a constituent of numerous important compounds found in living cells, including amino acids, proteins (enzymes), nucleic acids, and chlorophyll. 1.5% to 2% of plant dry matter is nitrogen and approximately 16% of total plant protein. Thus, nitrogen availability is a major limiting factor for crop plant growth and production (Frink et al. (1999) Proc Natl Acad Sci USA 96(4): 1175-1180), and has as well a major impact on protein accumulation and amino acid composition. Therefore, of great interest are crop plants with increased seed yield-related traits, when grown under nitrogen-limiting conditions.

[0228] In one embodiment performance of the methods of the invention gives plants grown under conditions of reduced nutrient availability, particularly under conditions of reduced nitrogen availability, having increased seed yield-related traits relative to control plants grown under comparable conditions. Therefore, according to the present invention, there is provided a method for increasing seed yield-related traits in plants grown under conditions of reduced nutrient availability, preferably reduced nitrogen availability, which method comprises increasing expression in a plant of a nucleic acid sequence encoding an AHL19/20 polypeptide. Reduced nutrient availability may result from a deficiency or excess of nutrients such as nitrogen, phosphates and other phosphorous-containing compounds, potassium, calcium, cadmium, magnesium, manganese, iron and boron, amongst others. Preferably, reduced nutrient availability is reduced nitrogen availability.

[0229] In one embodiment performance of the methods of the invention gives plants grown under conditions of reduced nutrient availability, particularly under conditions of reduced nitrogen availability, having enhanced yield-related traits relative to control plants grown under comparable conditions. Therefore, according to the present invention, there is provided a method for enhancing yield-related traits in plants grown under conditions of reduced nutrient availability, which method comprises increasing expression in a plant of a nucleic acid sequence encoding a GRP polypeptide. Reduced nutrient availability may comprise reduced availability of nutrients such as nitrogen, phosphates and other phosphorous-containing compounds, potassium, calcium, cadmium, magnesium, manganese, iron and boron, amongst

others.

[0230] Performance of the methods of the invention gives plants grown under non-stress conditions increased yield relative to control plants. Therefore, according to the present invention, there is provided a method for increasing yield in plants grown under non-stress conditions, which method comprises increasing expression in above ground plant parts of a nucleic acid encoding an AAT-like polypeptide.

[0231] The present invention encompasses plants or parts thereof (including seeds) or cells thereof obtainable by the methods according to the present invention. The plants or parts thereof or cells thereof comprise a nucleic acid transgene encoding a yield increasing polypeptide selected from the group consisting of: an AT-hook motif nuclear localized 19/20 (AHL19/20), GRP (Growth Regulating Protein, wherein said GRP polypeptide is a metallothionein 2a (MT2a) polypeptide), an alanine aminotransferase (AAT)-like polypeptide and an alanine aminotransferase (AAT) polypeptide as defined above.

[0232] The invention also provides genetic constructs and vectors to facilitate introduction and/or increased expression in plants of nucleic acid sequences encoding yield increasing polypeptides selected from the group consisting of: an AT-hook motif nuclear localized 19/20 (AHL19/20), GRP (Growth Regulating Protein, wherein said GRP polypeptide is a metallothionein 2a (MT2a) polypeptide), an alanine aminotransferase (AAT)-like polypeptide and an alanine aminotransferase (AAT) polypeptide. The gene constructs may be inserted into vectors, which may be commercially available, suitable for transforming into plants and for expression of the gene of interest in the transformed cells. The invention also provides use of a gene construct as defined herein in the methods of the invention.

[0233] More specifically, the present invention provides a construct comprising:

(a) a nucleic acid sequence encoding a yield increasing polypeptide selected from the group consisting of: an AT-hook motif nuclear localized 19/20 (AHL19/20), GRP (Growth Regulating Protein, wherein said GRP polypeptide is a metallothionein 2a (MT2a) polypeptide)" an alanine aminotransferase (AAT)-like polypeptide and an alanine aminotransferase (AAT) polypeptide as defined above;

(b) one or more control sequences capable of increasing expression of the nucleic acid sequence of (a); and optionally
(c) a transcription termination sequence.

[0234] In one embodiment, the nucleic acid sequence encoding an AHL19/20 polypeptide is as defined above. The term "control sequence" and "termination sequence" are as defined herein.

[0235] Preferably, one of the control sequences of a construct is a constitutive promoter isolated from a plant genome. An example of a plant constitutive promoter is a GOS2 promoter, preferably a rice GOS2 promoter, more preferably a GOS2 promoter as represented by SEQ ID NO: 35.

[0236] In one embodiment, the nucleic acid sequence encoding a GRP polypeptide is as defined above. The term "control sequence" and "termination sequence" are as defined herein.

[0237] Preferably, the nucleic acid encoding an AAT-like polypeptide or an AAT polypeptide is as defined above. The term "control sequence" and "termination sequence" are as defined herein.

[0238] Plants are transformed with a vector comprising any of the nucleic acid sequences described above. The skilled artisan is well aware of the genetic elements that must be present on the vector in order to successfully transform, select and propagate host cells containing the sequence of interest. The sequence of interest is operably linked to one or more control sequences (at least to a promoter).

[0239] Advantageously, any type of promoter, whether natural or synthetic, may be used to increase expression of the nucleic acid sequence. A constitutive promoter is particularly useful in the methods, preferably a constitutive promoter isolated from a plant genome. The plant constitutive promoter drives expression of a coding sequence at a level that is in all instances below that obtained under the control of a 35S CaMV viral promoter.

[0240] Other organ-specific promoters, for example for preferred expression in leaves, stems, tubers, meristems, seeds (embryo and/or endosperm), are useful in performing the methods of the invention. See the "Definitions" section herein for definitions of the various promoter types.

[0241] It should be clear that the applicability of the present invention is not restricted to a nucleic acid sequence encoding the AHL19/20 polypeptide, as represented by SEQ ID NO: 1, nor is the applicability of the invention restricted to expression of an AHL19/20 polypeptide-encoding nucleic acid sequence when driven by a constitutive promoter.

[0242] It should be clear that the applicability of the present invention is not restricted to the GRP polypeptide-encoding nucleic acid sequence represented by SEQ ID NO: 45, nor is the applicability of the invention restricted to expression of a GRP polypeptide-encoding nucleic acid sequence when driven by a constitutive promoter.

[0243] The constitutive promoter is preferably a GOS2 promoter, preferably a GOS2 promoter from rice. Further preferably the GOS2 promoter is represented by a nucleic acid sequence substantially similar to SEQ ID NO: 47, most preferably the GOS2 promoter is as represented by SEQ ID NO: 47. See Table 2 in the "Definitions" section herein for further examples of constitutive promoters.

[0244] It should be clear that the applicability of the present invention is not restricted to the AAT-like nucleic acid

represented by SEQ ID NO: 50, nor is the applicability of the invention restricted to expression of an AAT-like polypeptide-encoding nucleic acid when driven by a protochlorophyllid reductase promoter.

[0245] See the "Definitions" section herein for definitions of the various promoter types. Particularly useful in the methods of the invention is a root-specific promoter, particularly a root epidermis-specific promoter. The root-specific promoter is preferably a nitrate transporter promoter, further preferably from rice (Os NRT1 promoter as described by Lin, 2000). The promoter is represented by SEQ ID NO: 59. A nucleic acid sequence substantially similar to SEQ ID NO: 59 would also be useful in the methods of the invention. Examples of other root-specific promoters which may also be used to perform the methods of the invention are shown in Table 2b in the "Definitions" section above.

[0246] It should be clear that the applicability of the present invention is not restricted to the AAT nucleic acid represented by SEQ ID NO: 55, nor is the applicability of the invention restricted to expression of an AAT nucleic acid when driven by the rice nitrate transport promoter, OsNRT1.

[0247] Optionally, one or more terminator sequences may be used in the construct introduced into a plant. Additional regulatory elements may include transcriptional as well as translational increasers. Those skilled in the art will be aware of terminator and increaser sequences that may be suitable for use in performing the invention. An intron sequence may also be added to the 5' untranslated region (UTR) or in the coding sequence to increase the amount of the mature message that accumulates in the cytosol, as described in the definitions section. Other control sequences (besides promoter, increaser, silencer, intron sequences, 3'UTR and/or 5'UTR regions) may be protein and/or RNA stabilizing elements. Such sequences would be known or may readily be obtained by a person skilled in the art.

[0248] The genetic constructs of the invention may further include an origin of replication sequence that is required for maintenance and/or replication in a specific cell type. One example is when a genetic construct is required to be maintained in a bacterial cell as an episomal genetic element (e.g. plasmid or cosmid molecule). Preferred origins of replication include, but are not limited to, the f1-ori and colE1.

[0249] For the detection of the successful transfer of the nucleic acid sequences as used in the methods of the invention and/or selection of transgenic plants comprising these nucleic acid sequences, it is advantageous to use marker genes (or reporter genes). Therefore, the genetic construct may optionally comprise a selectable marker gene. Selectable markers are described in more detail in the "definitions" section herein.

[0250] The marker genes may be removed or excised from the transgenic cell once they are no longer needed. Techniques for marker removal are known in the art, useful techniques are described above in the definitions section.

[0251] It is known that upon stable or transient integration of nucleic acid sequences into plant cells, only a minority of the cells takes up the foreign DNA and, if desired, integrates it into its genome, depending on the expression vector used and the transfection technique used. To identify and select these integrants, a gene coding for a selectable marker (such as the ones described above) is usually introduced into the host cells together with the gene of interest. These markers can for example be used in mutants in which these genes are not functional by, for example, deletion by conventional methods. Furthermore, nucleic acid sequence molecules encoding a selectable marker can be introduced into a host cell on the same vector that comprises the sequence encoding the polypeptides of the invention or used in the methods of the invention, or else in a separate vector. Cells which have been stably transfected with the introduced nucleic acid sequence can be identified for example by selection (for example, cells which have integrated the selectable marker survive whereas the other cells die). The marker genes may be removed or excised from the transgenic cell once they are no longer needed. Techniques for marker gene removal are known in the art, useful techniques are described above in the definitions section.

[0252] In one embodiment the invention also provides a method for the production of transgenic plants having increased seed yield-related traits relative to control plants, comprising introduction and expression in a plant of any nucleic acid sequence encoding an AHL19/20 polypeptide as defined hereinabove.

[0253] More specifically, the present invention provides a method for the production of transgenic plants having increased seed yield-related traits relative to control plants, which method comprises:

- (i) introducing and expressing in a plant, plant part, or plant cell a nucleic acid sequence encoding an AHL19/20 polypeptide, under the control of plant constitutive promoter; and
- (ii) cultivating the plant cell, plant part or plant under conditions promoting plant growth and development.

[0254] The nucleic acid sequence of (i) may be any of the nucleic acid sequences capable of encoding an AHL19/20 polypeptide as defined herein.

[0255] In one embodiment the invention also provides a method for the production of transgenic plants grown under abiotic stress conditions having enhanced yield-related traits relative to control plants, comprising introduction and expression in a plant of a nucleic acid sequence encoding a GRP polypeptide as defined hereinabove.

[0256] More specifically, the present invention provides a method for the production of transgenic plants grown under abiotic stress conditions having enhanced yield-related traits relative to control plants, which method comprises:

1. introducing and expressing in a plant, plant part, or plant cell, a nucleic acid sequence encoding a GRP polypeptide; and
2. cultivating the plant, plant part or plant cell under conditions promoting plant growth and development.

[0257] The nucleic acid sequence of (i) may be any of the nucleic acid sequences capable of encoding a GRP polypeptide as defined herein.

[0258] In one embodiment the invention also provides a method for the production of transgenic plants having enhanced yield-related traits relative to control plants, comprising introduction and expression in above ground plant parts of any nucleic acid encoding an AAT-like polypeptide as defined hereinabove.

[0259] More specifically, the present invention provides a method for the production of transgenic plants having enhanced yield-related traits, which method comprises:

(i) introducing and expressing in above ground plant parts or in a plant cell an AAT-like nucleic acid under the control of a promoter active in above ground plant parts; and

(ii) cultivating the plant cell under conditions promoting plant growth and development.

[0260] The nucleic acid of (i) may be any of the AAT-like nucleic acids as defined herein.

[0261] More specifically, the present invention provides a method for the production of transgenic plants having increased yield-related traits, particularly increased (seed) yield, which method comprises:

(i) introducing and expressing in a plant or plant cell an AAT nucleic acid; and

(ii) cultivating the plant cell under non nitrogen limiting conditions.

[0262] The nucleic acid of (i) may be any of the nucleic acids capable of encoding an AAT polypeptide as defined herein.

[0263] The nucleic acid sequence may be introduced directly into a plant cell or into the plant itself (including introduction into a tissue, organ or any other part of a plant). According to a preferred feature of the present invention, the nucleic acid sequence is preferably introduced into a plant by transformation. The term "transformation" is described in more detail in the "definitions" section herein.

[0264] The genetically modified plant cells can be regenerated via all methods with which the skilled worker is familiar. Suitable methods can be found in the abovementioned publications by S.D. Kung and R. Wu, Potrykus or Höfgen and Willmitzer.

[0265] Generally after transformation, plant cells or cell groupings are selected for the presence of one or more markers which are encoded by plant-expressible genes co-transferred with the gene of interest, following which the transformed material is regenerated into a whole plant. To select transformed plants, the plant material obtained in the transformation is, as a rule, subjected to selective conditions so that transformed plants can be distinguished from untransformed plants. For example, the seeds obtained in the above-described manner can be planted and, after an initial growing period, subjected to a suitable selection by spraying. A further possibility consists in growing the seeds, if appropriate after sterilization, on agar plates using a suitable selection agent so that only the transformed seeds can grow into plants. Alternatively, the transformed plants are screened for the presence of a selectable marker such as the ones described above.

[0266] Following DNA transfer and regeneration, putatively transformed plants may also be evaluated, for instance using Southern analysis, for the presence of the gene of interest, copy number and/or genomic organisation. Alternatively or additionally, expression levels of the newly introduced DNA may be monitored using Northern and/or Western analysis, both techniques being well known to persons having ordinary skill in the art.

[0267] The generated transformed plants may be propagated by a variety of means, such as by clonal propagation or classical breeding techniques. For example, a first generation (or T1) transformed plant may be selfed and homozygous second-generation (or T2) transformants selected, and the T2 plants may then further be propagated through classical breeding techniques. The generated transformed organisms may take a variety of forms. For example, they may be chimeras of transformed cells and non-transformed cells; clonal transformants (e.g., all cells transformed to contain the expression cassette); grafts of transformed and untransformed tissues (e.g., in plants, a transformed rootstock grafted to an untransformed scion).

[0268] The present invention clearly extends to any plant cell or plant produced by any of the methods described herein, and to all plant parts and propagules thereof. The present invention extends further to encompass the progeny of a primary transformed or transfected cell, tissue, organ or whole plant that has been produced by any of the aforementioned methods, the only requirement being that progeny exhibit the same genotypic and/or phenotypic characteristic(s) as those produced by the parent in the methods according to the invention.

[0269] In one embodiment the invention also includes host cells containing an isolated nucleic acid sequence encoding an AHL19/20 polypeptide as defined hereinabove, operably linked to a plant constitutive promoter

[0270] In one embodiment the invention also includes host cells containing an isolated nucleic acid sequence encoding a GRP polypeptide as defined hereinabove.

[0271] In one embodiment the invention also includes host cells containing an isolated nucleic acid encoding an AAT-like polypeptide or an AAT polypeptide as defined hereinabove.

[0272] Preferred host cells according to the invention are plant cells. Host plants for the nucleic acids or the vector used in the method according to the invention, the expression cassette or construct or vector are, in principle, advantageously all plants, which are capable of synthesizing the polypeptides used in the inventive method.

[0273] The methods of the invention are advantageously applicable to any plant. Plants that are particularly useful in the methods of the invention include all plants, which belong to the superfamily Viridiplantae, in particular monocotyledonous and dicotyledonous plants including fodder or forage legumes, ornamental plants, food crops, trees or shrubs. According to a preferred embodiment of the present invention, the plant is a crop plant. Examples of crop plants include soybean, sunflower, canola, alfalfa, rapeseed, cotton, tomato, potato and tobacco. Further preferably, the plant is a monocotyledonous plant. Examples of monocotyledonous plants include sugarcane. More preferably the plant is a cereal. Examples of cereals include rice, maize, wheat, barley, millet, rye, triticale, sorghum and oats.

[0274] The invention also extends to harvestable parts of a plant comprising an isolated nucleic acid sequence encoding an AHL19/20 (as defined hereinabove) operably linked to a plant constitutive promoter, such as, but not limited to seeds, leaves, fruits, flowers, stems, rhizomes, tubers and bulbs. The invention furthermore relates to products derived, preferably directly derived, from a harvestable part of such a plant, such as dry pellets or powders, oil, fat and fatty acids, starch or proteins.

[0275] Methods for increasing expression of nucleic acid sequences or genes, or gene products, are well documented in the art and examples are provided in the definitions section.

[0276] As mentioned above, a preferred method for increasing expression of a nucleic acid sequence encoding an AHL19/20 polypeptide is by introducing and expressing in a plant a nucleic acid sequence encoding an AHL19/20 polypeptide; however the effects of performing the method, i.e. increasing seed yield-related traits, may also be achieved using other well known techniques, including but not limited to T-DNA activation tagging, TILLING, homologous recombination. A description of these techniques is provided in the definitions section.

[0277] As mentioned above, a preferred method for increasing expression of a nucleic acid sequence encoding a GRP polypeptide is by introducing and expressing in a plant a nucleic acid sequence encoding a GRP polypeptide; however the effects of performing the method, i.e. enhancing yield-related traits of plants grown under abiotic stress conditions, may also be achieved using other well known techniques, including but not limited to T-DNA activation tagging, TILLING, homologous recombination. A description of these techniques is provided in the definitions section.

[0278] As mentioned above, a preferred method for modulating (preferably, increasing) expression of a nucleic acid encoding an AAT-like polypeptide or an AAT polypeptide is by introducing and expressing in a plant a nucleic acid encoding an AAT-like polypeptide or an AAT polypeptide; however the effects of performing the method, i.e. enhancing yield-related traits may also be achieved using other well known techniques, including but not limited to T-DNA activation tagging, TILLING, homologous recombination. A description of these techniques is provided in the definitions section.

[0279] The present invention also encompasses use of nucleic acid sequences encoding AHL19/20 polypeptides as described herein and use of these AHL19/20 polypeptides in increasing any of the aforementioned seed yield-related traits in plants, under normal growth conditions and under conditions of reduced nutrient availability, preferably under conditions of reduced nitrogen availability.

[0280] The present invention also encompasses use of nucleic acid sequences encoding GRP polypeptides as described herein and use of these GRP polypeptides in enhancing any of the aforementioned yield-related traits in plants grown under abiotic stress conditions.

[0281] The present invention also encompasses use of nucleic acids encoding AAT-like polypeptides or AAT polypeptides as described herein and use of these AAT-like polypeptides or AAT polypeptides respectively in enhancing any of the aforementioned yield-related traits in plants.

[0282] Nucleic acids encoding yield increasing polypeptides described herein, or the yield increasing polypeptides themselves, may find use in breeding programmes in which a DNA marker is identified which may be genetically linked to a yield increasing polypeptide -encoding gene. The nucleic acids/genes, or the AAT-like polypeptides themselves may be used to define a molecular marker. This DNA or protein marker may then be used in breeding programmes to select plants having enhanced yield-related traits as defined hereinabove in the methods of the invention.

[0283] Allelic variants of a yield increasing polypeptide -encoding nucleic acid/gene may also find use in marker-assisted breeding programmes. Such breeding programmes sometimes require introduction of allelic variation by mutagenic treatment of the plants, using for example EMS mutagenesis; alternatively, the programme may start with a collection of allelic variants of so called "natural" origin caused unintentionally. Identification of allelic variants then takes place, for example, by PCR. This is followed by a step for selection of superior allelic variants of the sequence in question and which give increased yield. Selection is typically carried out by monitoring growth performance of plants containing different allelic variants of the sequence in question. Growth performance may be monitored in a greenhouse or in the

field. Further optional steps include crossing plants in which the superior allelic variant was identified with another plant. This could be used, for example, to make a combination of interesting phenotypic features.

[0284] Nucleic acids encoding a yield increasing polypeptide may also be used as probes for genetically and physically mapping the genes that they are a part of, and as markers for traits linked to those genes. Such information may be useful in plant breeding in order to develop lines with desired phenotypes. Such use of nucleic acids coding for yield increasing polypeptides requires only a nucleic acid sequence of at least 15 nucleotides in length. The yield increasing polypeptide-encoding nucleic acids may be used as restriction fragment length polymorphism (RFLP) markers. Southern blots (Sambrook J, Fritsch EF and Maniatis T (1989) *Molecular Cloning, A Laboratory Manual*) of restriction-digested plant genomic DNA may be probed with the AAT-like nucleic acids. The resulting banding patterns may then be subjected to genetic analyses using computer programs such as MapMaker (Lander et al. (1987) *Genomics* 1: 174-181) in order to construct a genetic map. In addition, the nucleic acids may be used to probe Southern blots containing restriction endonuclease-treated genomic DNAs of a set of individuals representing parent and progeny of a defined genetic cross. Segregation of the DNA polymorphisms is noted and used to calculate the position of the yield increasing polypeptide-encoding nucleic acid in the genetic map previously obtained using this population (Botstein et al. (1980) *Am. J. Hum. Genet.* 32:314-331).

[0285] The production and use of plant gene-derived probes for use in genetic mapping is described in Bernatzky and Tanksley (1986) *Plant Mol. Biol. Reporter* 4: 37-41. Numerous publications describe genetic mapping of specific cDNA clones using the methodology outlined above or variations thereof. For example, F2 intercross populations, backcross populations, randomly mated populations, near isogenic lines, and other sets of individuals may be used for mapping. Such methodologies are well known to those skilled in the art.

[0286] The nucleic acid sequence probes may also be used for physical mapping (i.e., placement of sequences on physical maps; see Hoheisel et al. In: *Non-mammalian Genomic Analysis: A Practical Guide*, Academic press 1996, pp. 319-346, and references cited therein).

[0287] In another embodiment, the nucleic acid sequence probes may be used in direct fluorescence in situ hybridisation (FISH) mapping (Trask (1991) *Trends Genet.* 7:149-154). Although current methods of FISH mapping favour use of large clones (several kb to several hundred kb; see Laan et al. (1995) *Genome Res.* 5:13-20), improvements in sensitivity may allow performance of FISH mapping using shorter probes.

[0288] A variety of nucleic acid sequence amplification-based methods for genetic and physical mapping may be carried out using the nucleic acid sequences. Examples include allele-specific amplification (Kazazian (1989) *J. Lab. Clin. Med.* 11:95-96), polymorphism of PCR-amplified fragments (CAPS; Sheffield et al. (1993) *Genomics* 16:325-332), allele-specific ligation (Landegren et al. (1988) *Science* 241:1077-1080), nucleotide extension reactions (Sokolov (1990) *Nucleic acid sequence Res.* 18:3671), Radiation Hybrid Mapping (Walter et al. (1997) *Nat. Genet.* 7:22-28) and Happy Mapping (Dear and Cook (1989) *Nucleic acid sequence Res.* 17:6795-6807). For these methods, the sequence of a nucleic acid sequence is used to design and produce primer pairs for use in the amplification reaction or in primer extension reactions. The design of such primers is well known to those skilled in the art. In methods employing PCR-based genetic mapping, it may be necessary to identify DNA sequence differences between the parents of the mapping cross in the region corresponding to the instant nucleic acid sequence. This, however, is generally not necessary for mapping methods.

[0289] The methods according to the present invention result in plants having increased seed yield-related traits, as described hereinbefore. These traits may also be combined with other economically advantageous traits, such as further yield-increasing traits, tolerance to other abiotic and biotic stresses, traits modifying various architectural features and/or biochemical and/or physiological features.

[0290] The methods according to the present invention result in plants grown under abiotic stress conditions having enhanced yield-related traits, as described hereinbefore. These traits may also be combined with other economically advantageous traits, such as further yield-enhancing traits, tolerance to other abiotic and biotic stresses, traits modifying various architectural features and/or biochemical and/or physiological features.

In one embodiment the invention relates to subject matter summarized as follows:

Item 1: A method for increasing seed yield-related traits in plants relative to control plants, comprising increasing expression in a plant of a nucleic acid sequence encoding an AT-hook motif nuclear localized 19/20 (AHL19/20) polypeptide, which AHL19/20 polypeptide comprises a domain having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% or more amino acid sequence identity to a Conserved Domain (CD) as represented by SEQ ID NO: 36, and optionally selecting for plants having increased seed yield-related traits.

Item 2: Method according to item 1, wherein said AHL19/20 polypeptide comprises: (i) a motif having at least 75%, 80%, 85%, 90%, 95%, 98%, 99% or more amino acid sequence identity to an AT-hook motif as represented by SEQ ID NO: 37; and (ii) a domain having at least 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% or more amino acid sequence identity to a plant and prokaryote conserved (PPC) domain as represented by SEQ ID

NO: 38.

Item 3: Method according to item 1 or 2, wherein said AHL19/20 polypeptide comprises: (i) a nuclear localisation signal; (ii) an AT-hook DNA binding motif with an InterPro entry IPR014476; and (iii) a plant and prokaryote conserved (PPC) domain with an InterPro entry IPR005175.

Item 4: Method according to any preceding item, wherein said AHL19/20 polypeptide, when used in the construction of an AHL phylogenetic tree, such as the one depicted in Figure 1 or in Figure 2, clusters with the AHL19/20 group of polypeptides comprising the polypeptide sequence as represented by SEQ ID NO: 2, rather than with any other AHL group.

Item 5: Method according to any preceding item, wherein said AHL19/20 polypeptide has in increasing order of preference at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% or more amino acid sequence identity to the AHL19/20 polypeptide as represented by SEQ ID NO: 2 or to any of the polypeptide sequences given in Table A herein.

Item 6: Method according to any preceding item, wherein said nucleic acid sequence encoding an AHL19/20 polypeptide is represented by any one of the nucleic acid sequence SEQ ID NOs given in Table A or a portion thereof, or a sequence capable of hybridising with any one of the nucleic acid sequences SEQ ID NOs given in Table A.

Item 7: Method according to any preceding item, wherein said nucleic acid sequence encodes an orthologue or paralogue of any of the polypeptide sequence SEQ ID NOs given in Table A.

Item 8: Method according to any preceding item, wherein said increased expression is effected by any one or more of: T-DNA activation tagging, TILLING, or homologous recombination.

Item 9: Method according to any preceding item, wherein said increased expression is effected by introducing and expressing in a plant a nucleic acid sequence encoding an AHL19/20 polypeptide.

Item 10: Method according to any preceding item, wherein said increased seed yield-related trait is one or more of: (i) increased number of flowers per panicle; (ii) increased total seed weight per plant; (iii) increased number of filled seeds; or (iv) increased harvest index.

Item 11: Method according to any preceding item, wherein said increased seed yield-related traits occur in plants grown under conditions of reduced nutrient availability, preferably under conditions of reduced nitrogen availability, relative to control plants.

Item 12: Method according to item 11, wherein said increased seed yield-related trait is one or more of: (i) increased total seed yield per plant; (ii) increased number of filled seeds; or (iii) increased harvest index.

Item 13: Method according to any preceding item, wherein said nucleic acid sequence is operably linked to a constitutive promoter, preferably to a plant constitutive promoter, more preferably to a GOS2 promoter, most preferably to a GOS2 promoter from rice as represented by SEQ ID NO: 35.

Item 14: Method according to any preceding item, wherein said nucleic acid sequence encoding an AHL19/20 polypeptide is of plant origin, preferably from a dicotyledonous plant, further preferably from the family Brassicaceae, most preferably from *Arabidopsis thaliana*.

Item 15: Plants, parts thereof (including seeds), or plant cells obtainable by a method according to any preceding item, wherein said plant, part or cell thereof comprises an isolated nucleic acid transgene encoding an AHL19/20 polypeptide operably linked to a plant constitutive promoter.

Item 16: Construct comprising:

- (a) A nucleic acid sequence encoding an AHL19/20 polypeptide as defined in any one of items 1 to 5;
- (b) one or more control sequences capable of driving expression of the nucleic acid sequence of (a); and optionally
- (c) a transcription termination sequence.

Item 17: Construct according to item 16, wherein said control sequence is a plant constitutive promoter, preferably a GOS2 promoter, more preferably a GOS2 promoter as represented by SEQ ID NO: 35.

Item 18: Use of a construct according to items 16 or 17 in a method for making plants having increased seed yield-related traits relative to control plants, which increased seed yield-related traits are one or more of: (i) increased number of flowers per panicle; (ii) increased total seed weight per plant; (iii) increased number of filled seeds; or (iv) increased harvest index.

Item 19: Plant, plant part or plant cell transformed with a construct according to item 16 or 17.

Item 20: Method for the production of transgenic plants having increased seed yield-related traits relative to control plants, comprising:

- (i) introducing and expressing in a plant, plant part, or plant cell, a nucleic acid sequence encoding an AHL19/20 polypeptide as defined in any one of items 1 to 6, under the control of plant constitutive promoter; and
- (ii) cultivating the plant cell, plant part, or plant under conditions promoting plant growth and development.

Item 21: Transgenic plant having increased seed yield-related traits relative to control plants, resulting from increased expression of a nucleic acid sequence encoding an AHL19/20 polypeptide as defined in any one of items 1 to 5, operably linked to a plant constitutive promoter, or a transgenic plant cell or transgenic plant part derived from said transgenic plant. Item 22: Transgenic plant according to item 15, 19 or 21, wherein said plant is a crop plant or a monocot or a cereal, such as rice, maize, wheat, barley, millet, rye, triticale, sorghum and oats, or a transgenic plant cell derived from said transgenic plant.

Item 23: Harvestable parts comprising a nucleic acid sequence encoding an AHL19/20 polypeptide of a plant according to item 22, wherein said harvestable parts are preferably seeds.

Item 24: Products derived from a plant according to item 22 and/or from harvestable parts of a plant according to item 23.

Item 25: Use of a nucleic acid sequence encoding an AHL19/20 polypeptide as defined in any one of items 1 to 6 in increasing seed yield-related traits, comprising one or more of: (i) increased number of flowers per panicles; (ii) increased total seed weight per plant; (iii) increased number of filled seeds; or (iv) increased harvest index.

Item 26: Use according to item 25, wherein said increased seed yield-related traits occur under conditions of reduced nutrient availability, preferably under conditions of reduced nitrogen availability.

[0291] In one embodiment the invention relates to subject matter summarized as follows:

Item 27: A method for enhancing yield-related traits in plants grown under abiotic stress conditions relative to control plants, comprising increasing expression in a plant of a nucleic acid sequence encoding a GRP polypeptide, wherein said GRP polypeptide is a metallothionein 2a (MT2a) polypeptide as represented by SEQ ID NO: 46 or an orthologue, paralogue, or homologue thereof, and optionally selecting for plants grown under abiotic stress conditions having enhanced yield-related traits.

Item 28: A method according to item 27, wherein said GRP polypeptide as represented by SEQ ID NO: 46 and an orthologue, paralogue, or homologue thereof, have an InterPro entry IPR000347, described as plant metallothionein, family 15.

Item 29: Method according to item 27 or 28, wherein said GRP polypeptide has in increasing order of preference at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% or more amino acid sequence identity to the GRP polypeptide as represented by SEQ ID NO: 46.

Item 30: Method according to any preceding item 27 to 29, wherein said nucleic acid sequence encoding a GRP polypeptide is represented by the nucleic acid sequence of SEQ ID NO: 45 or a portion thereof, or a sequence capable of hybridising with the nucleic acid sequence of SEQ ID NO: 45 or a portion thereof.

Item 31: Method according to any preceding item 27 to 30 wherein said increased expression is effected by introducing

and expressing in a plant a nucleic acid sequence encoding said GRP polypeptide.

Item 32: Method according to any preceding item 27 to 31, wherein said abiotic stress is an osmotic stress, selected from one or more of the following: water stress, salt stress, oxidative stress and ionic stress.

Item 33: Method according to item 32, wherein said water stress is drought stress.

Item 34: Method according to item 32, wherein said ionic stress is salt stress.

Item 35: Method according to any preceding item 27 to 34, wherein said enhanced yield-related traits are one or more of: increased aboveground biomass, increased total seed yield per plant, increased number of filled seeds, increased total number of filled seeds, increased number of primary panicles and increased seed fill rate, relative to control plants.

Item 36: Method according to any preceding item 27 to 35, wherein said nucleic acid sequence is operably linked to a constitutive promoter, preferably to a GOS2 promoter, most preferably to a GOS2 promoter from rice.

Item 37: Method according to any preceding item 27 to 36, wherein said nucleic acid sequence encoding a GRP polypeptide is of plant origin, preferably from a dicotyledonous plant, further preferably from the family Brassicaceae, more preferably from *Arabidopsis thaliana*.

Item 38: Use of a nucleic acid sequence encoding a GRP polypeptide in enhancing yield-related traits in plants grown under abiotic stress conditions.

Item 39: Use of a nucleic acid sequence encoding a GRP polypeptide according to item 38, wherein said enhanced yield-related traits are selected from one or more of: increased aboveground biomass, increased total seed yield per plant, increased number of filled seeds, increased total number of filled seeds, increased number of primary panicles and increased seed fill rate, relative to control plants.

Item 40: Use of a nucleic acid sequence encoding a GRP polypeptide according to item 39, wherein said abiotic stress is an osmotic stress, selected from one or more of the following: water stress, salt stress, oxidative stress and ionic stress.

Item 41: Use of a nucleic acid sequence encoding a GRP polypeptide according to item 40, said water stress is drought stress.

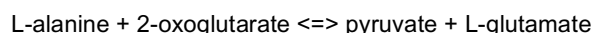
Item 42: Use of a nucleic acid sequence encoding a GRP polypeptide according to item 40, said ionic stress is salt stress.

[0292] In one embodiment the invention relates to subject matter summarized as follows:

Item 43: A method for enhancing yield-related traits in plants relative to control plants, comprising modulating expression in above ground plant parts of a nucleic acid encoding an alanine aminotransferase (AAT)-like polypeptide.

Item 44: Method according to item 43, wherein said AAT-like polypeptide comprises one or more of the following features:

(i) the ability to catalyse the following reaction:



(ii) belongs to enzyme classification code: EC 2.6.1.2.

(iii) has an amino transferase domain (referred to in InterPro by IPR004839; and in PFAM by PF00155)

(iv) has an 1-aminocyclopropane-1-carboxylate synthase domain (referred to in InterPro by IPR001176)

(v) is targeted to the mitochondria

(vi) when used in the construction of a phylogenetic tree containing AAT sequences, clusters with the group of AAT-like polypeptides comprising SEQ ID NO: 51 rather than with any other group of AATs or AAT-like se-

quences.

Item 45: Method according to item 43 or 44, wherein said modulated expression is effected by introducing and expressing in a plant a nucleic acid encoding an AAT-like polypeptide under the control of a promoter active in above ground plants parts.

Item 46: Method according to any preceding item 43 to 45, wherein said nucleic acid encoding an AAT-like polypeptide is capable of hybridising with the nucleic acid represented by SEQ ID NO: 50.

Item 47: Method according to any preceding item 43 to 46, wherein said nucleic acid sequence encodes an orthologue or paralogue of the protein represented by SEQ ID NO: 51.

Item 48: Method according to any preceding item 43 to 47, wherein said enhanced yield-related traits comprise increased yield, preferably increased seed yield relative to control plants.

Item 49: Method according to any preceding item 43 to 48, wherein said enhanced yield-related traits are obtained under non-stress conditions.

Item 50: Method according to any one of items 45 to 49, wherein said promoter active in above ground parts is a shoot-specific and/or leaf-specific promoter.

Item 51: Method according to any preceding item 43 to 50, wherein said nucleic acid encoding an AAT-like polypeptide is of algal origin, preferably from the genus *Chlamydomonas*, further preferably from the species *Chlamydomonas reinhardtii*.

Item 52: Plant or part thereof, including seeds, obtainable by a method according to any preceding item 43 to 51, wherein said plant or part thereof comprises a recombinant nucleic acid encoding an AAT-like polypeptide.

Item 53: Construct comprising:

- (a) nucleic acid encoding an AAT-like polypeptide as defined in any one of items 44, 46 or 47;
- (b) a promoter sequence capable of driving expression of the nucleic acid sequence of (a) in aboveground parts; and optionally
- (c) a transcription termination sequence.

Item 54: Use of a construct according to item 53 in a method for making plants having increased yield, particularly increased seed yield relative to control plants.

Item 55: Plant, plant part or plant cell transformed with a construct according to item 53.

Item 56: Method for the production of a transgenic plant having increased yield, particularly increased seed yield relative to control plants, comprising:

- (i) introducing and expressing in a plant a nucleic acid encoding an AAT-like polypeptide as defined in any one of items 44, 46 or 47, which nucleic acid is under the control of a promoter active in aboveground parts; and
- (ii) cultivating the plant cell under conditions promoting plant growth and development.

Item 57: Transgenic plant having increased yield, particularly increased seed yield, relative to control plants, resulting from increased expression of a nucleic acid encoding an AAT-like polypeptide as defined in any one of items 44, 46 or 47, which nucleic acid is under the control of a promoter active in above ground parts, or a transgenic plant cell derived from said transgenic plant.

Item 58: Transgenic plant according to item 52, 55 or 57, or a transgenic plant cell derived thereof, wherein said plant is a crop plant or a monocot or a cereal, such as rice, maize, wheat, barley, millet, rye, triticale, sorghum and oats.

Item 59: Harvestable parts of a plant according to item 58, wherein said harvestable parts are seeds.

Item 60: Products derived from a plant according to item 58 and/or from harvestable parts of a plant according to

item 59.

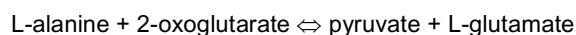
Item 61: Use of a nucleic acid encoding an AAT-like polypeptide, which nucleic acid is under the control of a promoter active in above ground parts, for increasing yield, particularly increasing seed yield in plants, relative to control plants.

[0293] In one embodiment the invention relates to subject matter summarized as follows:

Item 62: A method for enhancing yield-related traits in plants relative to control plants, comprising modulating expression in a plant of a nucleic acid encoding an alanine aminotransferase (AAT), which plants are grown under non limiting nitrogen availability. 63:

Item 63: Method according to item 62, wherein said AAT-like polypeptide comprises one or more of the following features:

(i) the ability to catalyse the following reaction:



(ii) belongs to enzyme classification code: EC 2.6.1.2.

(iii) has an amino transferase domain (referred to in InterPro by IPR004839; and in PFAM by PF00155)

(iv) has an 1-aminocyclopropane-1-carboxylate synthase domain (referred to in InterPro by IPR001176)

(v) when used in the construction of a phylogenetic tree containing AAT sequences, clusters with the group of AAT-like polypeptides comprising SEQ ID NO: 56 rather than with any other group of AATs or AAT-like sequences.

Item 64: Method according to item 62 or 63, wherein said modulated expression is effected by introducing and expressing in a plant a nucleic acid encoding an AAT-like polypeptide.

Item 65: Method according to any preceding item 62 to 64, wherein said nucleic acid encoding an AAT-like polypeptide is capable of hybridising with the nucleic acid represented by SEQ ID NO: 55.

Item 66: Method according to any preceding item 62 to 65, wherein said nucleic acid sequence encodes an orthologue or paralogue of the protein represented by SEQ ID NO: 56.

Item 67: Method according to any preceding item 62 to 66, wherein said enhanced yield-related traits comprise increased yield, preferably increased biomass and/or increased seed yield relative to control plants.

Item 68: Method according to any one of items 64 to 67, wherein said nucleic acid is operably linked to a tissue-specific promoter, preferably to a root-specific promoter, most preferably to a root-epidermis-specific promoter.

Item 69: Method according to item 68, wherein said root-epidermis-specific promoter is a nitrate transporter promoter, preferably from rice.

Item 70: Method according to any preceding item 62 to 69, wherein said nucleic acid encoding an AAT is of plant origin, preferably from a monocotyledonous plant, further preferably from the family Poaceae, more preferably from the genus *Oryza*, most preferably from *Oryza sativa*.

Item 71: Plant or part thereof, including seeds, obtainable by a method according to any preceding item 62 to 70, wherein said plant or part thereof comprises a recombinant nucleic acid encoding an AAT.

Item 72: Construct comprising:

(a) nucleic acid encoding an AAT as defined in item 63;

(b) a nitrate transporter promoter, preferably from rice; and optionally

(c) a transcription termination sequence.

Item 73: Use of a construct according to item 72 in a method for making plants having increased yield under non nitrogen limiting conditions, particularly increased biomass and/or increased seed yield relative to control plants.

Item 74: Plant, plant part or plant cell transformed with a construct according to item 71.

Item 75: Method for the production of a transgenic plant having increased yield under non nitrogen limiting conditions, particularly increased biomass and/or increased seed yield relative to control plants, comprising:

- (i) introducing and expressing in a plant a nucleic acid encoding an AAT as defined in item 63; and
- (ii) cultivating the plant cell under conditions promoting plant growth and development.

Item 76: Transgenic plant having increased yield under non nitrogen limiting conditions, particularly increased biomass and/or increased seed yield, relative to control plants, resulting from increased expression of a nucleic acid encoding an AAT as defined in item 63, or a transgenic plant cell derived from said transgenic plant.

Item 77: Transgenic plant according to items 71, 74 or 76, or a transgenic plant cell derived thereof, wherein said plant is a crop plant or a monocot or a cereal, such as rice, maize, wheat, barley, millet, rye, triticale, sorghum and oats.

Item 78: Harvestable parts of a plant according to item 77, wherein said harvestable parts are preferably shoot biomass and/or seeds.

Item 79: Products derived from a plant according to item 77 and/or from harvestable parts of a plant according to item 78.

Item 80: Use of a nucleic acid encoding an AAT in increasing yield under non nitrogen limiting conditions, particularly in increasing seed yield and/or shoot biomass in plants, relative to control plants.

Description of figures

[0294] The present invention will now be described with reference to the following figures in which:

Figure 1 represents a neighbour-joining tree constructed after an alignment of all the polypeptides belonging to the AHL family (described in Fujimoto et al., (2004) Plant Molec Biol, 56: 225-239) performed using the Clustal algorithm (1.83) of progressive alignment, using default values. The group of interest, comprising the two Arabidopsis paralogs AHL19 (SEQ ID NO: 2 or AT3G04570), and AHL20 (SEQ ID NO: 4 or AT4G14465) has been circled.

Figure 2 represents a neighbour-joining tree constructed after an alignment of all the polypeptides belonging to the AHL family (described in Fujimoto et al., (2004) Plant Molec Biol, 56: 225-239), and all the AHL19/20 orthologs and paralogs of Table A in Example 1 herein, performed using the Clustal algorithm (1.83) of progressive alignment, using default values.

Figure 3 represents a cartoon of an AHL19/20 polypeptide as represented by SEQ ID NO: 2, which comprises the following features: a predicted NLS, an AT-hook DNA binding motif (of which the core is the tripeptide GRP; comprised in InterPro entry IPR014476 (Predicted AT-hook DNA-binding)), a PPC domain (plant and prokaryotes conserved domain; comprised in InterPro entry IPR005175 (Protein of unknown function DUF296)), and Conserved Domain (CD) comprising both an AT-hook DNA binding motif and a PPC domain.

Figure 4 shows a CLUSTAL W (1.83) multiple sequence alignment of the Conserved Domain of AHL19/20 polypeptides from Table A (as represented by SEQ ID NO: 38 for SEQ ID NO: 2), where a number of features are identified. From the N-terminus to the C-terminus of the polypeptides, these are: (i) a predicted nuclear localisation signal (NLS); (ii) an AT-hook DNA binding motif, with the core tripeptide GRP; and (iii) a PPC domain (DUF 296).

Figure 5 shows the binary vector for increased expression in *Oryza sativa* of a nucleic acid sequence encoding an AHL19/20 polypeptide under the control of a GOS2 promoter (pGOS2) from rice.

Figure 6 details examples of sequences useful in performing the methods according to the present invention.

Fig. 7 represents the binary vector for increased expression in *Oryza sativa* of a GRP-encoding nucleic acid sequence (wherein said GRP polypeptide is a metallothionein 2a (MT2a) polypeptide) under the control of a rice GOS2 promoter (pGOS2::GRP)

Fig. 8 details examples of sequences useful in performing the methods according to the present invention.

Fig. 9 shows the binary vector for increased expression in *Oryza sativa* of an AAT-like nucleic acid under the control of a rice protochlorophyllid promoter.

Fig. 10 details examples of sequences useful in performing the methods according to the present invention.

Fig. 11 shows the binary vector for increased expression in *Oryza sativa* of an AAT nucleic acid under the control of a rice OsNRT1 promoter.

Fig. 12 details examples of sequences useful in performing the methods according to the present invention.

Examples

[0295] The present invention will now be described with reference to the following examples, which are by way of illustration alone. The following examples are not intended to completely define or otherwise limit the scope of the invention.

[0296] DNA manipulation: unless otherwise stated, recombinant DNA techniques are performed according to standard protocols described in (Sambrook (2001) Molecular Cloning: a laboratory manual, 3rd Edition Cold Spring Harbor Laboratory Press, CSH, New York) or in Volumes 1 and 2 of Ausubel et al. (1994), Current Protocols in Molecular Biology, Current Protocols. Standard materials and methods for plant molecular work are described in Plant Molecular Biology Labfax (1993) by R.D.D. Croy, published by BIOS Scientific Publications Ltd (UK) and Blackwell Scientific Publications (UK).

Example 1: Identification of sequences related to the nucleic acid sequence used in the methods of the invention

[0297] Sequences (full length cDNA, ESTs or genomic) related to the nucleic acid sequence used in the methods of the present invention were identified amongst those maintained in the Entrez Nucleotides database at the National Center for Biotechnology Information (NCBI) using database sequence search tools, such as the Basic Local Alignment Tool (BLAST) (Altschul et al. (1990) J. Mol. Biol. 215:403-410; and Altschul et al. (1997) Nucleic Acids Res. 25:3389-3402). The program is used to find regions of local similarity between sequences by comparing nucleic acid sequence or polypeptide sequences to sequence databases and by calculating the statistical significance of matches. For example, the polypeptide encoded by the nucleic acid sequence of the present invention was used for the TBLASTN algorithm, with default settings and the filter to ignore low complexity sequences set off. The output of the analysis was viewed by pairwise comparison, and ranked according to the probability score (E-value), where the score reflect the probability that a particular alignment occurs by chance (the lower the E-value, the more significant the hit). In addition to E-values, comparisons were also scored by percentage identity. Percentage identity refers to the number of identical nucleotides (or amino acids) between the two compared nucleic acid sequence (or polypeptide) sequences over a particular length. In some instances, the default parameters may be adjusted to modify the stringency of the search. For example the E-value may be increased to show less stringent matches. This way, short nearly exact matches may be identified.

[0298] Table A provides a list of nucleic acid sequences related to the nucleic acid sequence used in the methods of the present invention.

Table A: Examples of AHL19/20 polypeptides:

Name	Source organism	Nucleic acid SEQ ID NO:	Polypeptide SEQ ID NO:	Database accession number	Status
Arath_AHL19	<i>Arabidopsis thaliana</i>	1	2	AT3G04570 NP_566232	Full Length (FL)
Arath_AHL20	<i>Arabidopsis thaliana</i>	3	4	AT4G14465 NP_567432	FL
Aqufo_AHL19/20	<i>Aquilegia formosa</i> x <i>Aquilegia pubescens</i>	5	6	contig of DT758489, DT758488.1	FL
Brana_AHL19/20	<i>Brassica napus</i>	7	8	CS226287	FL

(continued)

Name	Source organism	Nucleic acid SEQ ID NO:	Polypeptide SEQ ID NO:	Database accession number	Status
Brara_AHL19/20	<i>Brassica rapa</i>	9	10	AC189468	FL
Glyma_AHL19/20	<i>Glycine max</i>	11	12	CS137412	FL
Goshi_AHL19/20	<i>Gossypium hirsutum</i>	13	14	DW519458	FL
Lacsa_AHL19/20	<i>Lactuca sativa</i>	15	16	DW047323	FL
Lotja_AHL19/20	<i>Lotus japonicus</i>	17	18	AP004971	FL
Orysa_AHL19/20	<i>Oryza sativa</i>	19	20	AK110263 Os08g056320 0	FL
Orysa_AHL19/20 II	<i>Oryza sativa</i>	21	22	CT837915 Os02g0820800	FL
Poptr_AHL19/20	<i>Populus tremuloides</i>	23	24	scaff_XIII.441	FL
Soltu_AHL19/20	<i>Solanum tuberosum</i>	25	26	Contig of CN215397.1 CK276075.1	FL
Thlca_AHL19/20	<i>Thlaspi caerulescens</i>	27	28	DQ022564	FL
Vitvi_AHL19/20	<i>Vitis vinifera</i>	29	30	AM463589	FL
Vitvi_AHL19/20 II	<i>Vitis vinifera</i>	31	32	AM429692	FL
Zeama_AHL19/20	<i>Zea mays</i>	33	34	AC190270	FL

[0299] In some instances, related sequences have tentatively been assembled and publicly disclosed by research institutions, such as The Institute for Genomic Research (TIGR). The Eukaryotic Gene Orthologs (EGO) database may be used to identify such related sequences, either by keyword search or by using the BLAST algorithm with the nucleic acid sequence or polypeptide sequence of interest. On other instances, special nucleic acid sequence databases have been created for particular organisms, such as by the Joint Genome Institute, for example for poplar and *Ostreococcus tauri*.

Example 2: Alignment of polypeptide sequences of the invention

[0300] Alignment of polypeptide sequences is performed using the AlignX programme from the Vector NTI package (Invitrogen) which is based on the popular ClustalW algorithm of progressive alignment (Thompson et al. (1997) Nucleic Acids Res 25:4876-4882; Chenna et al. (2003). Nucleic Acids Res 31:3497-3500). Default values are for the gap open penalty of 10, for the gap extension penalty of 0.1 and the selected weight matrix is Blosom 62 (if polypeptides are aligned). Minor manual editing may be done to further optimise the alignment. A phylogenetic tree of polypeptides is constructed using a neighbour-joining clustering algorithm as provided in the AlignX programme from Vector NTI (Invitrogen).

[0301] Alignment of all the *Arabidopsis thaliana* AHL polypeptide sequences identified in Fujimoto *et al.* (2004; Table A1 below) was performed using the Clustal algorithm (1.83) of progressive alignment, with default values (Thompson et al. (1997) Nucleic Acids Res 25:4876-4882; Chenna et al. (2003). Nucleic Acids Res 31:3497-3500). A neighbour-joining tree was constructed thereafter, and is represented in Figure 1 of the present application. The group of interest, comprising the two *Arabidopsis* paralogs AHL19 (SEQ ID NO: 2 or AT3G04570), and AHL20 (SEQ ID NO: 4 or AT4G14465) has been circled. Any polypeptide falling within this AHL19/20 group (after a new multiple alignment step as described hereinabove) is considered to be useful in performing the methods of the invention as described herein.

Table A1: AHL polypeptides identified in *Arabidopsis thaliana*

AHL number	Tair accession number	NCBI accession number
AHL1	At4g12080	NP_192945

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(continued)

5
10
15
20
25
30
35
40
45

AHL number	Tair accession number	NCBI accession number
AHL2	At4g22770	NP_194008
AHL3	At4g25320	NP_194262
AHL4	At5g51590	NP_199972
AHL5	At1g63470	NP_176536
AHL6	At5g62260	NP_201032
AHL7	At4g00200	NP_191931
AHL8	At5g46640	NP_199476
AHL9	At2g45850	NP_182109
AHL10	At2g33620	NP_565769
AHL11	At3g61310	NP_191690
AHL12	At1g63480	NP_176537
AHL13	At4g17950	NP_567546
AHL14	At3g04590	NP_187109
AHL15	At3g55560	NP_191115
AHL16	At2g42940	NP_181822
AHL17	At5g49700	NP_199781
AHL18	At3g60870	NP_191646
AHL19	At3g04570	NP_566232
AHL20	At4g 14465	NP_567432
AHL21	At2g35270	NP_181070
AHL22	At2g45430	NP_182067
AHL23	At4g17800	NP_193515
AHL24	At4g22810	NP_194012
AHL25	At4g35390	NP_195265
AHL26	At4g12050	NP_192942
AHL27	At1g20900	NP_173514
AHL28	At1g14490	NP_172901
AHL29	At1g76500	NP_177776

[0302] A second multiple sequence alignment was performed including all of the AHL19/20 orthologous polypeptides of Table A and all of the AHL sequences of Table A1. A neighbour-joining tree was constructed thereafter, and is represented in Figure 2 of the present application. The group of interest, comprising the two *Arabidopsis* paralogs AHL19 (SEQ ID NO: 2 or AT3G04570), and AHL20 (SEQ ID NO: 4 or AT4G14465) has been circled. Any polypeptide falling within this AHL19/20 group is considered to be useful in performing the methods of the invention as described herein.

[0303] The Conserved Domain (CD) of SEQ ID NO: 2, as represented by SEQ ID NO: 36, was identified after multiple sequence alignment of the AHL19/20 polypeptides of Table A. In a second step, the CDs of the AHL19/20 polypeptides of Table A were selected (out of the full length polypeptide sequence) and aligned, using the Clustal algorithm (1.83) of progressive alignment, using default values. A number of features can be identified, and are marked in Figure 4. From the N-terminus to the C-terminus of the polypeptides, these are: (i) a predicted nuclear localisation signal (NLS); (ii) an AT-hook DNA binding motif, with the core tripeptide GRP; and (iii) a PPC domain (DUF 296).

[0304] A phylogenetic tree of AAT-like polypeptides and AAT polypeptides is constructed using a neighbour-joining

clustering algorithm as provided in the AlignX programme from the Vector NTI (Invitrogen).

Example 3: Calculation of global percentage identity between polypeptide sequences useful in performing the methods of the invention

[0305] Global percentages of similarity and identity between full length polypeptide sequences useful in performing the methods of the invention were determined using one of the methods available in the art, the MatGAT (Matrix Global Alignment Tool) software (BMC Bioinformatics. 2003 4:29. MatGAT: an application that generates similarity/identity matrices using protein or DNA sequences. Campanella JJ, Bitincka L, Smalley J; software hosted by Ledion Bitincka). MatGAT software generates similarity/identity matrices for DNA or protein sequences without needing pre-alignment of the data. The program performs a series of pair-wise alignments using the Myers and Miller global alignment algorithm (with a gap opening penalty of 12, and a gap extension penalty of 2), calculates similarity and identity using for example Blosom 62 (for polypeptides), and then places the results in a distance matrix. Sequence similarity is shown in the bottom half of the dividing line and sequence identity is shown in the top half of the diagonal dividing line.

[0306] Parameters used in the comparison were:

Scoring matrix: Blosom62

First Gap: 12

Extending gap: 2

[0307] A MATGAT table for local alignment of a specific domain, or data on % identity/similarity between specific domains may also be generated.

[0308] Results of the software analysis are shown in Table B for the global similarity and identity over the full length of the polypeptide sequences (excluding the partial polypeptide sequences). Percentage identity is given above the diagonal and percentage similarity is given below the diagonal.

[0309] The percentage identity between the polypeptide sequences useful in performing the methods of the invention can be as low as 52 % amino acid identity compared to SEQ ID NO: 2.

Table B: MatGAT results for global similarity and identity over the full length of the polypeptide sequences.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1. Aqufo_AHL19_20		63	64	61	61	60	73	66	71	59	61	66	70	63	73	71	58
2. Arath_AHL19	72		56	94	94	56	59	61	59	48	55	61	63	97	61	57	52
3. Arath_AHL20	76	70		54	54	56	63	63	61	54	58	59	61	57	61	63	54
4. Brana_AHL19_20	70	96	67		100	56	59	61	58	49	55	60	61	94	60	56	52
5. Brara_AHL19_20	70	96	67	100		56	59	61	58	49	55	60	61	94	60	56	52
6. Glyma_AHL19_20	75	68	73	68	68		60	64	59	52	58	66	63	58	67	59	55
7. Goshi_AHL19_20	82	69	75	67	67	77		64	70	61	66	64	67	59	71	79	61
8. Lacsa_AHL19_20	79	72	77	72	72	81	80		63	56	59	69	73	61	72	64	56
9. Lotja_AHL19_20	82	72	75	71	71	74	80	77		59	61	63	69	58	71	72	62
10. Orysa_AHL19_20	67	58	65	58	58	65	68	69	66		66	52	55	48	56	62	70
11. Orysa_AHL19_20II	71	64	69	63	63	73	76	73	72	70		59	60	56	60	66	67
12. Poptr_AHL19_20	81	71	73	71	71	80	80	81	80	64	74		72	61	75	62	54
13. Soltu_AHL19_20	82	75	76	75	75	79	77	81	80	61	70	87		63	78	66	58
14. Thlca_AHL19_20	73	98	69	95	95	70	68	72	73	58	66	72	75		61	58	52
15. Vitvi_AHL19_20	85	72	74	71	71	81	79	82	82	65	70	85	90	71		69	57
16. Vitvi_AHL19_20II	80	66	74	66	66	73	84	78	79	70	76	75	76	66	78		63

[0310] The percentage identity can be substantially increased if the identity calculation is performed between the Conserved Domain (CD) of SEQ ID NO: 2 (as represented by SEQ ID NO: 36) and the CDs of the polypeptides useful in performing the invention. A CD comprises an AT-hook DNA binding motif (as represented by SEQ ID NO: 37 for SEQ

ID NO: 2) and a PPC domain (as represented by SEQ ID NO: 38 for SEQ ID NO: 2). Percentage identity over the CDs amongst the polypeptide sequences useful in performing the methods of the invention ranges between 75 % and 99% amino acid identity, as shown in Table B1. This is significantly higher than the percentage amino acid identity calculated between the full length AHL19/20 polypeptide sequences.

Table B1: MatGAT results for global similarity and identity over the CDs domain amongst of the polypeptide sequences.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1. CD_Aqufo_AHL19_20		81	81	80	80	77	92	83	87	86	87	81	84	81	84	92	86
2. CD_Arath_AHL19	91		78	98	98	75	82	77	78	76	80	78	80	99	81	81	77
3. CD_Arath_AHL20	93	88		77	77	73	84	80	79	77	81	76	79	78	79	82	78
4. CD_Brana_AHL19_20	91	99	88		100	75	82	77	78	76	79	78	79	98	80	80	78
5. CD_Brara_AHL19_20	91	99	88	100		75	82	77	78	76	79	78	79	98	80	80	78
6. CD_Glyma_AHL19_20	92	88	89	88	88		80	78	80	74	79	81	78	75	82	80	78
7. CD_Goshi_AHL19_20	98	91	93	91	91	93		86	92	87	89	85	88	82	90	94	88
8. CD_Lacsa_AHL19_20	96	90	92	90	90	92	96		81	78	83	86	87	77	88	83	78
9. CD_Lotja_AHL19_20	96	90	93	90	90	94	98	95		86	86	81	86	78	86	94	89
10. CD_Orysa_AHL19_20	94	87	89	87	87	89	92	92	93		89	78	84	76	81	86	95
11. CD_Orysa_AHL19_20II	94	88	89	88	88	93	95	93	96	95		81	87	80	86	89	90
12. CD_Poptr_AHL19_20	96	88	91	88	88	93	97	95	96	92	93		86	78	87	84	78
13. CD_Soltu_AHL19_20	94	89	91	89	89	93	96	95	96	90	93	96		80	90	89	83
14. CD_Thlca_AHL19_20	91	100	88	99	99	88	91	90	90	87	88	89	89		81	81	77
15. CD_Vitvi_AHL19_20	94	90	91	90	90	94	96	96	96	90	93	95	96	90		88	81
16. CD_Vitvi_AHL19_20II	96	90	92	90	90	93	98	94	99	92	95	95	95	90	95		87
17. CD_Zeama_AHL19_20	94	88	90	88	88	91	94	93	95	96	96	92	90	88	90	93	

Example 4: Identification of domains comprised in polypeptide sequences useful in performing the methods of the invention

[0311] The Integrated Resource of Protein Families, Domains and Sites (InterPro) database is an integrated interface for the commonly used signature databases for text- and sequence-based searches. The InterPro database combines these databases, which use different methodologies and varying degrees of biological information about well-characterized proteins to derive protein signatures. Collaborating databases include SWISS-PROT, PROSITE, TrEMBL, PRINTS, ProDom and Pfam, Smart and TIGRFAMs. Interpro is hosted at the European Bioinformatics Institute in the United Kingdom.

[0312] The results of the InterPro scan of the polypeptide sequence as represented by SEQ ID NO: 2 are presented in Table C.

Table C: InterPro scan results of the polypeptide sequence as represented by SEQ ID NO: 2

InterPro accession number and name	Integrated database name	Integrated database accession number	Integrated database accession name	Amino acid coordinates on SEQ ID NO: 2
IPR005175 Domain: Protein of unknown function DUF296	PFAM	PF03479	DUF 296	107-232
InterPro IPR014476 Family: Predicted AT-hook DNA-binding motif	PIR	PIRSF016021	ESCAROLA	1-315

[0313] The GRP polypeptide sequences are used as query to search the InterPro database. GRP polypeptides useful in performing the methods of the invention match one InterPro entry, as seen in the table below:

InterPro accession number	Integrated database name	Integrated database accession number	Integrated database accession name
InterPro IPR000347 Plant metallothionein, family 15	ProDom	PD001611	Metallothionein_15p
	Pfam	PF01439	Metallothionein_2

[0314] The results of the InterPro scan of the polypeptide sequence as represented by SEQ ID NO: 51 and SEQ ID NO: 56 are presented below
InterPro:

IPR001176: domain 1-aminocyclopropane-1-carboxylate synthase, region [203-224][256-280][292-315]
IPR004839: domain Aminotransferase, class I and II, region [145-314]

PFAM:

PF00155 domain Aminotransferase class I and II, with score 8.4e-19, region [108-509]

Example 5: Subcellular localisation prediction of the polypeptide sequences useful in performing the methods of the invention

[0315] TargetP 1.1 predicts the subcellular location of eukaryotic proteins. The location assignment is based on the predicted presence of any of the N-terminal pre-sequences: chloroplast transit peptide (cTP), mitochondrial targeting peptide (mTP) or secretory pathway signal peptide (SP). Scores on which the final prediction is based are not really probabilities, and they do not necessarily add to one. However, the location with the highest score is the most likely according to TargetP, and the relationship between the scores (the reliability class) may be an indication of how certain the prediction is. The reliability class (RC) ranges from 1 to 5, where 1 indicates the strongest prediction. TargetP is maintained at the server of the Technical University of Denmark.

[0316] For the sequences predicted to contain an N-terminal presequence a potential cleavage site can also be predicted.

[0317] A number of parameters were selected, such as organism group (non-plant or plant), cutoff sets (none, predefined set of cutoffs, or user-specified set of cutoffs), and the calculation of prediction of cleavage sites (yes or no).

[0318] The results of TargetP 1.1 analysis of the polypeptide sequence as represented by SEQ ID NO: 2 are presented Table D7. The "plant" organism group has been selected, and no cutoffs defined. The predicted subcellular localization of the polypeptide sequence as represented by SEQ ID NO: 2 is not chloroplastic, not mitochondrial and not the secretory pathway, but most likely the nucleus.

[0319] A predicted nuclear localisation signal (NLS) is found (by multiple sequence alignment, followed by eye inspection, for example) in the AHL19/20 polypeptide of SEQ ID NO: 2. An NLS is one or more short sequences of positively charged lysines or arginines. SEQ ID NO: 2 of the present invention is predicted to localise to the nuclear compartment of eucaryotic cells.

Table D: TargetP 1.1 analysis of the polypeptide sequence as represented by SEQ ID NO: 2

Length (AA)	315
Chloroplastic transit peptide	0.100
Mitochondrial transit peptide	0.278

(continued)

Secretory pathway signal peptide	0.033
Other subcellular targeting	0.703
Predicted Location	Other
Reliability class	3

[0320] The results of TargetP 1.1 analysis of the polypeptide sequence as represented by SEQ ID NO: 51 are presented below.

[0321] TargetP prediction: mitochondrial (0.837, quality 2)

[0322] Many algorithms can be used to perform subcellular localisation prediction analyses, including:

- ChloroP 1.1 hosted on the server of the Technical University of Denmark;
- Protein Prowler Subcellular Localisation Predictor version 1.2 hosted on the server of the Institute for Molecular Bioscience, University of Queensland, Brisbane, Australia;
- PENCE Proteome Analyst PA-GOSUB 2.5 hosted on the server of the University of Alberta, Edmonton, Alberta, Canada;
- TMHMM, hosted on the server of the Technical University of Denmark;

Example 6: Assay related to the polypeptide sequences useful in performing the methods of the invention

[0323] The AAT-like polypeptide may be able to catalyse the following reaction:

L-alanine + 2-oxoglutarate pyruvate + L-glutamate

A person skilled in the art will be readily able to check for such activity.

Example 7: Cloning of nucleic acid sequence of the invention

[0324] Unless otherwise stated, recombinant DNA techniques are performed according to standard protocols described in (Sambrook (2001) Molecular Cloning: a laboratory manual, 3rd Edition Cold Spring Harbor Laboratory Press, CSH, New York) or in Volumes 1 and 2 of Ausubel et al. (1994), Current Protocols in Molecular Biology, Current Protocols. Standard materials and methods for plant molecular work are described in Plant Molecular Biology Labfax (1993) by R.D.D. Croy, published by BIOS Scientific Publications Ltd (UK) and Blackwell Scientific Publications (UK).

[0325] The *Arabidopsis thaliana* cDNA encoding the AHL19 polypeptide was amplified by PCR using as template an Arabidopsis cDNA bank synthesized from mRNA extracted from mixed plant tissues. Primer prm8135 (SEQ ID NO: 41; sense, 5'-ggggacaagttgtacaaaaagcaggcttaacaatggcgaatccatggtg-3') and primer prm08136 (SEQ ID NO: 42; reverse, complementary, 5'-ggggaccactttgtacaagaaagctgggttaaaaccattttaacgcacg-3'), which include the AttB sites for Gateway recombination, were used for PCR amplification. PCR was performed using Hifi Taq DNA polymerase in standard conditions. A PCR fragment of the expected length (including attB sites) was amplified and purified also using standard methods. The first step of the Gateway procedure, the BP reaction, was then performed, during which the PCR fragment recombined in vivo with the pDONR201 plasmid to produce, according to the Gateway terminology, an "entry clone". Plasmid pDONR201 was purchased from Invitrogen, as part of the Gateway® technology.

Cloning of SEQ ID NO: 45:

[0326] The nucleic acid sequence SEQ ID NO: 45 used in the methods of the invention was amplified by PCR using as template a custom-made Arabidopsis thaliana mixed tissues cDNA library (in pCMV Sport 6.0; Invitrogen, Paisley, UK). PCR was performed using Hifi Taq DNA polymerase in standard conditions, using 200 ng of template in a 50 µl PCR mix. The primers used were prm03240 (SEQ ID NO: 48; sense:

5' ggggacaagttgtacaaaaagcaggcttcacaatgtctgtgtggaggaa 3'

and prm03241 (SEQ ID NO: 49; reverse, complementary):

5' ggggaccactttgtacaagaaagctgggttcacttgagggtgcaag 3',

which include the AttB sites for Gateway recombination. The amplified PCR fragment was purified also using standard methods. The first step of the Gateway procedure, the BP reaction, was then performed, during which the PCR fragment recombines *in vivo* with the pDONR201 plasmid to produce, according to the Gateway terminology, an "entry clone". Plasmid pDONR201 was purchased from Invitrogen, as part of the Gateway® technology.

[0327] The nucleic acid sequence SEQ ID NO: 50 used in the methods of the invention was amplified by PCR using Hifi Taq DNA polymerase in standard conditions, using 200 ng of template in a 50 µl PCR mix. The primers used were prm08408 (SEQ ID NO: 53; sense, start codon in bold):

5'-ggggacaagttgtacaaaaagcaggcttaacaatgcggaaggaagcgac-3'

and prm08409 (SEQ ID NO: 54; reverse, complementary):

5'-ggggaccactttgtacaagaaagctgggtcgaattgctaagctgttacga-3',

which include the AttB sites for Gateway recombination. The amplified PCR fragment was purified also using standard methods. The first step of the Gateway procedure, the BP reaction, was then performed, during which the PCR fragment recombines *in vivo* with the pDONR201 plasmid to produce, according to the Gateway terminology, an "entry clone", pAAT-like. Plasmid pDONR201 was purchased from Invitrogen, as part of the Gateway® technology.

[0328] The nucleic acid sequence SEQ ID NO: 55 used in the methods of the invention was amplified by PCR using as template an *Oryza sativa* cDNA library (in pCMV Sport 6.0; Invitrogen, Paisley, UK). PCR was performed using Hifi Taq DNA polymerase in standard conditions, using 200 ng of template in a 50 µl PCR mix. The primers used were prm001646 (SEQ ID NO: 58; sense, start codon in bold):

5'-ggggacaagttgtacaaaaagcaggcttcacaatggctgtcccagc-3'

and prm001647 (SEQ ID NO: 59; reverse, complementary):

5'-ggggaccactttgtacaagaaagctgggtaattcagtcgcggtacg-3',

which include the AttB sites for Gateway recombination. The amplified PCR fragment was purified also using standard methods. The first step of the Gateway procedure, the BP reaction, was then performed, during which the PCR fragment recombines *in vivo* with the pDONR201 plasmid to produce, according to the Gateway terminology, an "entry clone", pATT. Plasmid pDONR201 was purchased from Invitrogen, as part of the Gateway® technology.

Example 8: Expression vector construction using the nucleic acid sequence as disclosed

[0329] The entry clone comprising SEQ ID NO: 1 was subsequently used in an LR reaction with a destination vector used for *Oryza sativa* transformation. This vector contained as functional elements within the T-DNA borders: a plant selectable marker; a screenable marker expression cassette; and a Gateway cassette intended for LR *in vivo* recombination with the nucleic acid sequence of interest already cloned in the entry clone. A rice GOS2 promoter (SEQ ID NO: 35) for constitutive expression was located upstream of this Gateway cassette.

[0330] After the LR recombination step, the resulting expression vector pGOS2::AHL19/20 and (Figure 5) was transformed into *Agrobacterium* strain LBA4044 according to methods well known in the art.

[0331] The entry clone comprising SEQ ID NO: 45 was then used in an LR reaction with a destination vector used for *Oryza sativa* transformation. This vector contained as functional elements within the T-DNA borders: a plant selectable marker; a screenable marker expression cassette; and a Gateway cassette intended for LR *in vivo* recombination with the nucleic acid sequence of interest already cloned in the entry clone. A rice HMGB promoter (SEQ ID NO: 47) for constitutive expression was located upstream of this Gateway cassette.

[0332] After the LR recombination step, the resulting expression vector pGOS2::GRP (Figure 7) was transformed into *Agrobacterium* strain LBA4044 according to methods well known in the art.

[0333] The entry clone comprising SEQ ID NO: 50 was then used in an LR reaction with a destination vector used for *Oryza sativa* transformation. This vector contained as functional elements within the T-DNA borders: a plant selectable marker; a screenable marker expression cassette; and a Gateway cassette intended for LR *in vivo* recombination with the nucleic acid sequence of interest already cloned in the entry clone. A rice putative protochlorophyllid reductase promoter (SEQ ID NO: 52) for shoot and leaf-specific expression was located upstream of this Gateway cassette.

[0334] After the LR recombination step, the resulting expression vector (Figure 9) was transformed into *Agrobacterium* strain LBA4044 according to methods well known in the art.

[0335] The entry clone comprising SEQ ID NO: 55 was then used in an LR reaction with a destination vector used for

Oryza sativa transformation. This vector contained as functional elements within the T-DNA borders: a plant selectable marker; a screenable marker expression cassette; and a Gateway cassette intended for LR *in vivo* recombination with the nucleic acid sequence of interest already cloned in the entry clone. A rice NRT1 promoter (SEQ ID NO: 57) for root epidermis- and root hair-specific expression was located upstream of this Gateway cassette.

[0336] After the LR recombination step, the resulting expression vector pNRT1::ATT (Figure 11) was transformed into *Agrobacterium* strain LBA4044 according to methods well known in the art.

Example 9: Plant transformation

Rice transformation

[0337] The *Agrobacterium* containing the expression vectors were used independently to transform *Oryza sativa* plants. Mature dry seeds of the rice japonica cultivar Nipponbare were dehusked. Sterilization was carried out by incubating for one minute in 70% ethanol, followed by 30 minutes in 0.2% HgCl₂, followed by a 6 times 15 minutes wash with sterile distilled water. The sterile seeds were then germinated on a medium containing 2,4-D (callus induction medium). After incubation in the dark for four weeks, embryogenic, scutellum-derived calli were excised and propagated on the same medium. After two weeks, the calli were multiplied or propagated by subculture on the same medium for another 2 weeks. Embryogenic callus pieces were subcultured on fresh medium 3 days before co-cultivation (to boost cell division activity).

[0338] *Agrobacterium* strain LBA4404 containing each individual expression vector was used independently for co-cultivation. *Agrobacterium* was inoculated on AB medium with the appropriate antibiotics and cultured for 3 days at 28°C. The bacteria were then collected and suspended in liquid co-cultivation medium to a density (OD₆₀₀) of about 1. The suspension was then transferred to a Petri dish and the calli immersed in the suspension for 15 minutes. The callus tissues were then blotted dry on a filter paper and transferred to solidified, co-cultivation medium and incubated for 3 days in the dark at 25°C. Co-cultivated calli were grown on 2,4-D-containing medium for 4 weeks in the dark at 28°C in the presence of a selection agent. During this period, rapidly growing resistant callus islands developed. After transfer of this material to a regeneration medium and incubation in the light, the embryogenic potential was released and shoots developed in the next four to five weeks. Shoots were excised from the calli and incubated for 2 to 3 weeks on an auxin-containing medium from which they were transferred to soil. Hardened shoots were grown under high humidity and short days in a greenhouse.

[0339] Approximately 35 independent T0 rice transformants were generated for each construct. The primary transformants were transferred from a tissue culture chamber to a greenhouse. After a quantitative PCR analysis to verify copy number of the T-DNA insert, only single copy transgenic plants that exhibit tolerance to the selection agent were kept for harvest of T1 seed. Seeds were then harvested three to five months after transplanting. The method yielded single locus transformants at a rate of over 50 % (Aldemita and Hodges1996, Chan et al. 1993, Hiei et al. 1994).

Example 10: Phenotypic evaluation procedure

10.1 Evaluation setup

[0340] Approximately 35 independent T0 rice transformants were generated. The primary transformants were transferred from a tissue culture chamber to a greenhouse for growing and harvest of T1 seed. Six events, of which the T1 progeny segregated 3:1 for presence/absence of the transgene, were retained. For each of these events, approximately 10 T1 seedlings containing the transgene (hetero- and homo-zygotes) and approximately 10 T1 seedlings lacking the transgene (nullizygotes) were selected by monitoring visual marker expression. The transgenic plants and the corresponding nullizygotes were grown side-by-side at random positions. Greenhouse conditions were of short days (12 hours light), 28°C in the light and 22°C in the dark, and a relative humidity of 70%.

[0341] From the stage of sowing until the stage of maturity the plants were passed several times through a digital imaging cabinet. At each time point digital images (2048x1536 pixels, 16 million colours) were taken of each plant from at least 6 different angles.

Reduced nutrient (nitrogen) availability screen

[0342] Plants from six events (T2 seeds) were grown in potting soil under normal conditions except for the nutrient solution. The pots were watered from transplantation to maturation with a specific nutrient solution containing reduced N nitrogen (N) content, usually between 7 to 8 times less. The rest of the cultivation (plant maturation, seed harvest) was the same as for plants not grown under abiotic stress. Growth and yield parameters were recorded as detailed for growth under normal conditions.

10.2 Statistical analysis: F-test

[0343] A two factor ANOVA (analysis of variants) was used as a statistical model for the overall evaluation of plant phenotypic characteristics. An F-test was carried out on all the parameters measured of all the plants of all the events transformed with the gene of the present invention. The F-test was carried out to check for an effect of the gene over all the transformation events and to verify for an overall effect of the gene, also known as a global gene effect. The threshold for significance for a true global gene effect was set at a 5% probability level for the F-test. A significant F-test value points to a gene effect, meaning that it is not only the mere presence or position of the gene that is causing the differences in phenotype.

[0344] Four T1 events were further evaluated in the T2 generation following the same evaluation procedure as for the T1 generation but with more individuals per event.

[0345] When two experiments with overlapping events were carried out, a combined analysis was performed. This is useful to check consistency of the effects over the two experiments, and if this is the case, to accumulate evidence from both experiments in order to increase confidence in the conclusion. The method used is a mixed-model approach that takes into account the multilevel structure of the data (i.e. experiment - event - segregants). P values were obtained by comparing likelihood ratio test to chi square distributions.

[0346] From the stage of sowing until the stage of maturity the plants were passed several times through a digital imaging cabinet. At each time point digital images (2048x1536 pixels, 16 million colours) were taken of each plant from at least 6 different angles.

Drought screen in case of GRP polypeptide

[0347] Rice plants from T1, T2 or further generations were grown in potting soil under normal conditions until they approached the heading stage. They were then transferred to a "dry" section where irrigation was withheld. Humidity probes were inserted in randomly chosen pots to monitor the soil water content (SWC). When SWC went below certain thresholds, the plants were automatically re-watered continuously until a normal level was reached again. The plants were then re-transferred again to normal conditions. The rest of the cultivation (plant maturation, seed harvest) was the same as for plants not grown under abiotic stress conditions. Growth and yield parameters were recorded as detailed for growth under normal conditions.

Salt stress screen in case of GRP polypeptide

[0348] Rice plants from T1, T2 or further generations were grown on a substrate made of coco fibers and argex (3 to 1 ratio). A normal nutrient solution was used during the first two weeks after transplanting the plantlets in the greenhouse. After the first two weeks, 25 mM of salt (NaCl) was added to the nutrient solution, until the plants were harvested. Growth and yield parameters were recorded as detailed for growth under normal conditions.

Drought screen in case of AAT-like polypeptide and AAT polypeptide

[0349] Plants from T2 seeds were grown in potting soil under normal conditions until they approached the heading stage. They were then transferred to a "dry" section where irrigation was withheld. Humidity probes were inserted in randomly chosen pots to monitor the soil water content (SWC). When SWC went below certain thresholds, the plants were automatically re-watered continuously until a normal level was reached again. The plants were then re-transferred again to normal conditions. The rest of the cultivation (plant maturation, seed harvest) was the same as for plants not grown under abiotic stress conditions. Growth and yield parameters are recorded as detailed for growth under normal conditions.

Nitrogen use efficiency screen in case of AAT-like polypeptide and AAT polypeptide

[0350] Rice plants from T2 seeds were grown in potting soil under normal conditions except for the nutrient solution. The pots were watered from transplantation to maturation with a specific nutrient solution containing reduced N nitrogen (N) content, usually between 7 to 8 times less. The rest of the cultivation (plant maturation, seed harvest) was the same as for plants not grown under abiotic stress. Growth and yield parameters are recorded as detailed for growth under normal conditions.

10.3 Parameters measured

Biomass-related parameter measurement

[0351] From the stage of sowing until the stage of maturity the plants were passed several times through a digital imaging cabinet. At each time point digital images (2048x1536 pixels, 16 million colours) were taken of each plant from at least 6 different angles.

[0352] The plant aboveground area (or leafy biomass) was determined by counting the total number of pixels on the digital images from aboveground plant parts discriminated from the background. This value was averaged for the pictures taken on the same time point from the different angles and was converted to a physical surface value expressed in square mm by calibration. Experiments show that the aboveground plant area measured this way correlates with the biomass of plant parts above ground. The above ground area is the area measured at the time point at which the plant had reached its maximal leafy biomass. The early vigour is the plant (seedling) aboveground area three weeks post-germination. Increase in root biomass is expressed as an increase in total root biomass (measured as maximum biomass of roots observed during the lifespan of a plant); or as an increase in the root/shoot index (measured as the ratio between root mass and shoot mass in the period of active growth of root and shoot).

[0353] Early vigour was determined by counting the total number of pixels from aboveground plant parts discriminated from the background. This value was averaged for the pictures taken on the same time point from different angles and was converted to a physical surface value expressed in square mm by calibration. The results concerning early vigour described below are for plants three weeks post-germination.

Seed-related parameter measurements

[0354] The mature primary panicles were harvested, counted, bagged, barcode-labelled and then dried for three days in an oven at 37°C. The panicles were then threshed and all the seeds were collected and counted. The filled husks were separated from the empty ones using an air-blowing device. The empty husks were discarded and the remaining fraction was counted again. The filled husks were weighed on an analytical balance. The number of filled seeds was determined by counting the number of filled husks that remained after the separation step. The total seed weight per plant was measured by weighing all filled husks harvested from one plant. Total seed number per plant was measured by counting the number of husks harvested from a plant. Thousand Kernel Weight (TKW) is extrapolated from the number of filled seeds counted and their total weight. The Harvest Index (HI) in the present invention is defined as the ratio between the total seed weight per plant and the above ground area (mm²), multiplied by a factor 10⁶. The total number of flowers per panicle as defined in the present invention is the ratio between the total number of seeds and the number of mature primary panicles. The seed fill rate as defined in the present invention is the proportion (expressed as a %) of the number of filled seeds over the total number of seeds (or florets).

Example 11: Results of the phenotypic evaluation of the transgenic rice plants under normal growth conditions

[0355] The results of the evaluation of transgenic rice plants expressing the nucleic acid sequence encoding an AHL19/20 polypeptide as represented by SEQ ID NO: 2, under the control of the GOS2 promoter for constitutive expression, and grown under normal growth conditions, are presented below.

[0356] There was a significant increase in the number of flowers per panicle, in the total seed yield per plant, in the total number of filled seeds, and in the harvest index of the transgenic plants compared to corresponding nullizygotes (controls), as shown in Table E.

Table E: Results of the evaluation of transgenic rice plants expressing the nucleic acid sequence encoding an AHL19/20 polypeptide as represented by SEQ ID NO: 2, under the control of the GOS2 promoter for constitutive expression, under normal growth conditions.

Trait	Average % increase in 6 events in the T1 generation
Number of flowers per panicles	14%
Total seed yield per plant	17%
Total number of filled seeds	17%
Harvest index	17%

[0357] The evaluation of transgenic rice plants grown under non-stress conditions and expressing an AAT-like nucleic acid under the control of a protochlorophyllid reductase promoter from rice showed a significant increase in Harvest Index (HI) for transgenic plants compared to control plants. An increase in early vigour, total seed weight and in the number of filled seeds was also observed in transgenic plants compared to control plants.

[0358] The evaluation of transgenic rice plants grown under non nitrogen limiting conditions and expressing an AAT nucleic acid under the control of an NRT1 promoter from rice showed an increase in above ground area, plant height, early vigour compared to control plants.

Example 12: Results of the phenotypic evaluation of the transgenic rice plants under reduced nutrient (nitrogen) availability growth conditions

[0359] The results of the evaluation of transgenic rice plants expressing the nucleic acid sequence encoding an AHL19/20 polypeptide as represented by SEQ ID NO: 2, under the control of the GOS2 promoter for constitutive expression, and grown under reduced nutrient (nitrogen) availability growth conditions, are presented below.

[0360] There was a significant increase in the total seed yield per plant, in the total number of filled seeds, and in the harvest index of the transgenic plants compared to corresponding nullizygotes (controls), as shown in Table F.

Table F: Results of the evaluation of transgenic rice plants expressing the nucleic acid sequence encoding an AHL19/20 polypeptide as represented by SEQ ID NO: 2, under the control of the GOS2 promoter for constitutive expression, under reduced nutrient (nitrogen) availability growth conditions.

Trait	Average % increase in 2 events in the T1 generation
Early vigor	18%
Total seed yield per plant	26%
Total number of filled seeds	27%

(continued)

Trait	Average % increase in 2 events in the T1 generation
Total number of seeds	24%

Example 13: Results of the phenotypic evaluation of the transgenic rice plants under salt and/or drought stress growth conditions

[0361] The transgenic rice plants expressing the GRP nucleic acid sequence represented by SEQ ID NO: 45 under control of the GOS2 promoter, growing under salt stress conditions, showed an increase of more than 5% for aboveground biomass, total seed yield per plant, number of filled seeds, total number of seeds and number of first panicles, relative to control plants grown under comparable conditions, as shown in the Table below.

	Overall average % increase in the T2 generation
Aboveground biomass	20%
Total seed yield per plant	32%
Number of filled seeds	29%
Total number of seeds	19%
Number of first panicles	23%

[0362] The transgenic rice plants expressing the GRP nucleic acid sequence represented by SEQ ID NO: 45 under control of the GOS2 promoter, growing under drought stress conditions, showed an increase of more than 5% for aboveground biomass, total seed yield per plant, number of filled seeds, total number of seeds, and seed fill rate, relative to control plants grown under comparable conditions, as shown in the Table below.

	Average % increase for best event in the T1 generation
Total seed yield per plant	39%
Number of filled seeds	38%
Total number of seeds	19%
Seed fill rate	12%

Example 14: Examples of transformation of other crops

Corn transformation

[0363] Transformation of maize (*Zea mays*) is performed with a modification of the method described by Ishida et al. (1996) Nature Biotech 14(6): 745-50. Transformation is genotype-dependent in corn and only specific genotypes are

amenable to transformation and regeneration. The inbred line A188 (University of Minnesota) or hybrids with A188 as a parent are good sources of donor material for transformation, but other genotypes can be used successfully as well. Ears are harvested from corn plant approximately 11 days after pollination (DAP) when the length of the immature embryo is about 1 to 1.2 mm. Immature embryos are cocultivated with *Agrobacterium tumefaciens* containing the expression vector, and transgenic plants are recovered through organogenesis. Excised embryos are grown on callus induction medium, then maize regeneration medium, containing the selection agent (for example imidazolinone but various selection markers can be used). The Petri plates are incubated in the light at 25 °C for 2-3 weeks, or until shoots develop. The green shoots are transferred from each embryo to maize rooting medium and incubated at 25 °C for 2-3 weeks, until roots develop. The rooted shoots are transplanted to soil in the greenhouse. T1 seeds are produced from plants that exhibit tolerance to the selection agent and that contain a single copy of the T-DNA insert.

Wheat transformation

[0364] Transformation of wheat is performed with the method described by Ishida et al. (1996) Nature Biotech 14(6): 745-50. The cultivar Bobwhite (available from CIMMYT, Mexico) is commonly used in transformation. Immature embryos are co-cultivated with *Agrobacterium tumefaciens* containing the expression vector, and transgenic plants are recovered through organogenesis. After incubation with *Agrobacterium*, the embryos are grown *in vitro* on callus induction medium, then regeneration medium, containing the selection agent (for example imidazolinone but various selection markers can be used). The Petri plates are incubated in the light at 25 °C for 2-3 weeks, or until shoots develop. The green shoots are transferred from each embryo to rooting medium and incubated at 25 °C for 2-3 weeks, until roots develop. The rooted shoots are transplanted to soil in the greenhouse. T1 seeds are produced from plants that exhibit tolerance to the selection agent and that contain a single copy of the T-DNA insert.

Soybean transformation

[0365] Soybean is transformed according to a modification of the method described in the Texas A&M patent US 5,164,310. Several commercial soybean varieties are amenable to transformation by this method. The cultivar Jack (available from the Illinois Seed foundation) is commonly used for transformation. Soybean seeds are sterilised for *in vitro* sowing. The hypocotyl, the radicle and one cotyledon are excised from seven-day old young seedlings. The epicotyl and the remaining cotyledon are further grown to develop axillary nodes. These axillary nodes are excised and incubated with *Agrobacterium tumefaciens* containing the expression vector. After the cocultivation treatment, the explants are washed and transferred to selection media. Regenerated shoots are excised and placed on a shoot elongation medium. Shoots no longer than 1 cm are placed on rooting medium until roots develop. The rooted shoots are transplanted to soil in the greenhouse. T1 seeds are produced from plants that exhibit tolerance to the selection agent and that contain a single copy of the T-DNA insert.

Rapeseed/canola transformation

[0366] Cotyledonary petioles and hypocotyls of 5-6 day old young seedling are used as explants for tissue culture and transformed according to Babic et al. (1998, Plant Cell Rep 17: 183-188). The commercial cultivar Westar (Agriculture Canada) is the standard variety used for transformation, but other varieties can also be used. Canola seeds are surface-sterilized for *in vitro* sowing. The cotyledon petiole explants with the cotyledon attached are excised from the *in vitro* seedlings, and inoculated with *Agrobacterium* (containing the expression vector) by dipping the cut end of the petiole explant into the bacterial suspension. The explants are then cultured for 2 days on MSBAP-3 medium containing 3 mg/l BAP, 3 % sucrose, 0.7 % Phytagar at 23 °C, 16 hr light. After two days of co-cultivation with *Agrobacterium*, the petiole explants are transferred to MSBAP-3 medium containing 3 mg/l BAP, cefotaxime, carbenicillin, or timentin (300 mg/l) for 7 days, and then cultured on MSBAP-3 medium with cefotaxime, carbenicillin, or timentin and selection agent until shoot regeneration. When the shoots are 5 - 10 mm in length, they are cut and transferred to shoot elongation medium (MSBAP-0.5, containing 0.5 mg/l BAP). Shoots of about 2 cm in length are transferred to the rooting medium (MS0) for root induction. The rooted shoots are transplanted to soil in the greenhouse. T1 seeds are produced from plants that exhibit tolerance to the selection agent and that contain a single copy of the T-DNA insert.

Alfalfa transformation

[0367] A regenerating clone of alfalfa (*Medicago sativa*) is transformed using the method of (McKersie et al., 1999 Plant Physiol 119: 839-847). Regeneration and transformation of alfalfa is genotype dependent and therefore a regenerating plant is required. Methods to obtain regenerating plants have been described. For example, these can be selected from the cultivar Rangelander (Agriculture Canada) or any other commercial alfalfa variety as described by Brown DCW

and A Atanassov (1985. Plant Cell Tissue Organ Culture 4: 111-112). Alternatively, the RA3 variety (University of Wisconsin) has been selected for use in tissue culture (Walker et al., 1978 Am J Bot 65:654-659). Petiole explants are cocultivated with an overnight culture of *Agrobacterium tumefaciens* C58C1 pMP90 (McKersie et al., 1999 Plant Physiol 119: 839-847) or LBA4404 containing the expression vector. The explants are cocultivated for 3 d in the dark on SH induction medium containing 288 mg/L Pro, 53 mg/L thioproline, 4.35 g/L K₂SO₄, and 100 µM acetosyringone. The explants are washed in half-strength Murashige-Skoog medium (Murashige and Skoog, 1962) and plated on the same SH induction medium without acetosyringone but with a suitable selection agent and suitable antibiotic to inhibit *Agrobacterium* growth. After several weeks, somatic embryos are transferred to BOi2Y development medium containing no growth regulators, no antibiotics, and 50 g/L sucrose. Somatic embryos are subsequently germinated on half-strength Murashige-Skoog medium. Rooted seedlings were transplanted into pots and grown in a greenhouse. T1 seeds are produced from plants that exhibit tolerance to the selection agent and that contain a single copy of the T-DNA insert.

Cotton transformation

[0368] Cotton (*Gossypium hirsutum* L.) transformation is performed using *Agrobacterium tumefaciens*, on hypocotyls explants. The commercial cultivars such as Coker 130 or Coker 312 (SeedCo, Lubbock, TX) are standard varieties used for transformation, but other varieties can also be used. The seeds are surface sterilized and germinated in the dark. Hypocotyl explants are cut from the germinated seedlings to lengths of about 1-1.5 centimeter. The hypocotyl explant is submersed in the *Agrobacterium tumefaciens* inoculum containing the expression vector, for 5 minutes then cocultivated for about 48 hours on MS +1.8 mg/l KNO₃ + 2% glucose at 24° C, in the dark. The explants are transferred the same medium containing appropriate bacterial and plant selectable markers (renewed several times), until embryogenic calli is seen. The calli are separated and subcultured until somatic embryos appear. Plantlets derived from the somatic embryos are matured on rooting medium until roots develop. The rooted shoots are transplanted to potting soil in the greenhouse. T1 seeds are produced from plants that exhibit tolerance to the selection agent and that contain a single copy of the T-DNA insert.

Example 13: Examples of abiotic stress screens

Drought screen

[0369] Plants from a selected number of events are grown in potting soil under normal conditions until they approached the heading stage. They are then transferred to a "dry" section where irrigation is withheld. Humidity probes are inserted in randomly chosen pots to monitor the soil water content (SWC). When SWC go below certain thresholds, the plants are automatically re-watered continuously until a normal level is reached again. The plants are then re-transferred to normal conditions. The rest of the cultivation (plant maturation, seed harvest) is the same as for plants not grown under abiotic stress conditions. Growth and yield parameters are recorded as detailed for growth under normal conditions.

Salt stress screen

[0370] Plants are grown on a substrate made of coco fibers and argex (3 to 1 ratio). A normal nutrient solution is used during the first two weeks after transplanting the plantlets in the greenhouse. After the first two weeks, 25 mM of salt (NaCl) is added to the nutrient solution, until the plants were harvested. Growth and yield parameters are recorded as detailed for growth under normal conditions.

Example 14: Abiotic stress screens

Nitrogen use efficiency screen

[0371] Rice plants from T1, T2 or further generations are grown in potting soil under normal conditions except for the nutrient solution. The pots are watered from transplantation to maturation with a specific nutrient solution containing reduced N nitrogen (N) content, usually between 7 to 8 times less. The rest of the cultivation (plant maturation, seed harvest) is the same as for plants not grown under abiotic stress. Growth and yield parameters are recorded as detailed for growth under normal conditions.

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 gaagatgagg acagagaaaa cagcgacgag ccaagagagg gagctattga cgtcgccacc 180
 acgcgcggcc ctaggggacg tccaccgggc tccagaaaca agccgaaacc gccgatattc 240
 45 gtcaccggag acagccctaa cgcgctgcgg agccacgtca tggagattgc cgtcggagcc 300
 gacatcgccg actgctgggc gcagttcgct cggaggcgcc agcgcggggt ttccattctc 360
 agcggcagcg ggaccgtcgt caacgtcaat ctccggcaac ccacggcacc cggcgccgtc 420
 atggcgctcc acggccgctt cgacatcctc tccctcaccg gtcctttct ccctgggccc 480
 tcccctcccg gcgccaccgg gctcacaatc tacctcgccg gaggccaggg gcagatcgtc 540
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 <210> 12
 <211> 280

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<212> PRT
<213> Glycine max

<400> 12

5	Met	Ala	Asn	Arg	Trp	Trp	Thr	Gly	Ser	Val	Gly	Leu	Glu	Asn	Ser	Gly
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	His	Ser	Met	Lys	Lys	Pro	Asp	Leu	Gly	Phe	Ser	Met	Asn	Glu	Ser	Thr
				20					25					30		
	Val	Thr	Gly	Asn	His	Ile	Gly	Glu	Glu	Asp	Glu	Asp	Arg	Glu	Asn	Ser
			35					40					45			
10	Asp	Glu	Pro	Arg	Glu	Gly	Ala	Ile	Asp	Val	Ala	Thr	Arg	Arg	Pro	
	50					55					60					
	Arg	Gly	Arg	Pro	Pro	Gly	Ser	Arg	Asn	Lys	Pro	Lys	Pro	Pro	Ile	Phe
	65					70				75					80	
	Val	Thr	Arg	Asp	Ser	Pro	Asn	Ala	Leu	Arg	Ser	His	Val	Met	Glu	Ile
				85						90				95		
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				100					105					110		
	Arg	Gln	Arg	Gly	Val	Ser	Ile	Leu	Ser	Gly	Ser	Gly	Thr	Val	Val	Asn
			115					120					125			
	Val	Asn	Leu	Arg	Gln	Pro	Thr	Ala	Pro	Gly	Ala	Val	Met	Ala	Leu	His
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						150					155				160	
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				165						170					175	
25	Gly	Gln	Ile	Val	Gly	Gly	Gly	Val	Val	Gly	Pro	Leu	Val	Ala	Ala	Gly
				180					185					190		
	Pro	Val	Leu	Val	Met	Ala	Ala	Thr	Phe	Ser	Asn	Ala	Thr	Tyr	Glu	Arg
			195					200					205			
	Leu	Pro	Leu	Glu	Asp	Asp	Asp	Gln	Glu	Gln	His	Gly	Gly	Gly	Gly	Gly
			210				215					220				
30	Gly	Gly	Ser	Pro	Gln	Glu	Lys	Thr	Gly	Gly	Pro	Gly	Glu	Ala	Ser	Ser
						230					235				240	
	Ser	Ile	Ser	Val	Tyr	Asn	Asn	Asn	Val	Pro	Pro	Ser	Leu	Gly	Leu	Pro
				245						250					255	
	Asn	Gly	Gln	His	Leu	Asn	His	Glu	Ala	Tyr	Ser	Ser	Pro	Trp	Gly	His
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			275					280								

<210> 13
<211> 834
<212> DNA
<213> Gossypium hirsutum

<400> 13

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45	ggcgatgagc	ctaaagaagg	agcggtcgag	gtcggtaacc	gaagaccccg	aggtcgtcca	180
	ccgggatcca	aaaacaagcc	taaaccaccc	atttttgtga	caagggatag	ccctaacgcg	240
	ctccgtagtc	atgttatgga	agtcgcaagt	ggaaccgatg	tagccgagag	tatagcccaa	300
	ttcgctcgga	gaagacaacg	tggagtgtgt	ttgcttagcg	gcagcggctc	ggtcgccaac	360
	gttactctaa	gacaaccggc	agcaccggc	gcggtgggtg	cccttcatgg	aaggtttgaa	420
	attttgtctt	tgaccggggc	ttttctcccc	ggaccggctc	caccgggatc	gacagggctc	480
50	accgtgtact	tagctggttg	tcaaggacaa	gttggtggag	gaagtgttgt	cggctcactt	540
	atagcagcag	ggcctgttat	ggtcattgca	gcaacttttt	ccaacgcaac	ttatgaaaga	600
	ctgccttttag	aagatgaaga	agaagtgtga	agcgccgggtc	acggtggacc	gatgcaaggc	660
	ggagcaaacg	attcaccgcc	ggaaattggg	agtagcggag	gcggcgggtc	acacacaggt	720
	ctgcctgatc	catcttcact	tccaatatac	aatttgccctc	ctaatttact	ctcaaattgga	780
55	gggcaactag	ggcatgaacc	ctatggttgg	acacatggga	gaccacccta	ttaa	834

<210> 14

<211> 277
 <212> PRT
 <213> *Gossypium hirsutum*

5 <400> 14
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 1 5 10 15
 Ile Ser Met Asn Asp Ala Asn Lys Ser Arg Ser Asn Gly Arg Gly Asp
 20 25 30
 10 Asp Asp Asp Glu Asp Arg Asp Thr Gly Asp Glu Pro Lys Glu Gly Ala
 35 40 45
 Val Glu Val Gly Asn Arg Arg Pro Arg Gly Arg Pro Pro Gly Ser Lys
 50 55 60
 Asn Lys Pro Lys Pro Pro Ile Phe Val Thr Arg Asp Ser Pro Asn Ala
 65 70 75 80
 15 Leu Arg Ser His Val Met Glu Val Ala Ser Gly Thr Asp Val Ala Glu
 85 90 95
 Ser Ile Ala Gln Phe Ala Arg Arg Arg Gln Arg Gly Val Cys Leu Leu
 100 105 110
 Ser Gly Ser Gly Ser Val Ala Asn Val Thr Leu Arg Gln Pro Ala Ala
 115 120 125
 20 Pro Gly Ala Val Val Ala Leu His Gly Arg Phe Glu Ile Leu Ser Leu
 130 135 140
 Thr Gly Ala Phe Leu Pro Gly Pro Ala Pro Pro Gly Ser Thr Gly Leu
 145 150 155 160
 Thr Val Tyr Leu Ala Gly Gly Gln Gly Gln Val Val Gly Gly Ser Val
 165 170 175
 25 Val Gly Ser Leu Ile Ala Ala Gly Pro Val Met Val Ile Ala Ala Thr
 180 185 190
 Phe Ser Asn Ala Thr Tyr Glu Arg Leu Pro Leu Glu Asp Glu Glu Glu
 195 200 205
 Val Val Ser Ala Gly His Gly Gly Pro Met Gln Gly Gly Ala Asn Asp
 210 215 220
 30 Ser Pro Pro Glu Ile Gly Ser Ser Gly Gly Gly Gly Ser His Thr Gly
 225 230 235 240
 Leu Pro Asp Pro Ser Ser Leu Pro Ile Tyr Asn Leu Pro Pro Asn Leu
 245 250 255
 35 Leu Ser Asn Gly Gly Gln Leu Gly His Glu Pro Tyr Gly Trp Thr His
 260 265 270
 Gly Arg Pro Pro Tyr
 275

<210> 15
 <211> 813
 <212> DNA
 <213> *Lactuca sativa*

45 <400> 15
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 gcgatcaaga aaccagatct gggatatctca atgaatgata ccaccacagg aagtgaagaa 120
 gatgaaagag acaacaacag cgatgatcca agagaagggtg caattgaccc ttctaaccgt 180
 aggccacgag gccgacctcc gggatccaaa aacaaaccaa agccaccgat tttcgtcacc 240
 agagacagcc ctaacgccct ccgcagccac gtcattggagg tagcgagtgg tacagatatc 300
 gcagaaaagta tagctcaatt cagccgaaaa cgacaacgcg gtgtgtgtgt gatgagtgtc 360
 50 agcggcacag tcatgaatgt aaccctaaga caaccttcgg cacctggctc agtcatggct 420
 ctacaaggcc ggttcgagat tttatcccta accggtgcct tcttaccggg tccttctcct 480
 cctggatcca ccgggctcac tatatattta gctggtggcc agggccagggt tgtgggcgggt 540
 agcgtggtgg gatcattggt ggcacagga ccagtgatgg ttatagcagc caggttctcc 600
 aacgccacat atgaaagact cccggttgag gaagaggagg aagcagatac cgtgacacct 660
 gggctagggtg gtggtggatc accaccgcaa ctccgaatgg gtgatcagaa tccgatggca 720
 55 ggggtataata tgcagccgaa tttgatcccg aatggtggtg gacagatgaa ccatgaagct 780
 tttgctttgg ctcatggccg gccacgtac tag 813

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<210> 16
 <211> 270
 <212> PRT
 <213> Lactuca sativa

5

<400> 16
 Met Ser Asn Arg Trp Trp Thr Gly Gln Val Asn Val Ala Gly Val Glu
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 Thr Ser Ser Gln Ala Ile Lys Lys Pro Asp Leu Gly Ile Ser Met Asn
 20 25 30
 Asp Thr Thr Thr Gly Ser Glu Glu Asp Glu Arg Asp Asn Asn Ser Asp
 35 40 45
 Asp Pro Arg Glu Gly Ala Ile Asp Pro Ser Asn Arg Arg Pro Arg Gly
 50 55 60
 Arg Pro Pro Gly Ser Lys Asn Lys Pro Lys Pro Pro Ile Phe Val Thr
 65 70 75 80
 Arg Asp Ser Pro Asn Ala Leu Arg Ser His Val Met Glu Val Ala Ser
 85 90 95
 Gly Thr Asp Ile Ala Glu Ser Ile Ala Gln Phe Ser Arg Lys Arg Gln
 100 105 110
 Arg Gly Val Cys Val Met Ser Ala Ser Gly Thr Val Met Asn Val Thr
 115 120 125
 Leu Arg Gln Pro Ser Ala Pro Gly Ser Val Met Ala Leu Gln Gly Arg
 130 135 140
 Phe Glu Ile Leu Ser Leu Thr Gly Ala Phe Leu Pro Gly Pro Ser Pro
 145 150 155 160
 Pro Gly Ser Thr Gly Leu Thr Ile Tyr Leu Ala Gly Gly Gln Gly Gln
 165 170 175
 Val Val Gly Gly Ser Val Val Gly Ser Leu Val Ala Ser Gly Pro Val
 180 185 190
 Met Val Ile Ala Ala Thr Phe Ser Asn Ala Thr Tyr Glu Arg Leu Pro
 195 200 205
 Val Glu Glu Glu Glu Glu Ala Asp Thr Val Thr Pro Gly Leu Gly Gly
 210 215 220
 Gly Gly Ser Pro Pro Gln Leu Gly Met Gly Asp Gln Asn Pro Met Ala
 225 230 235 240
 Gly Tyr Asn Met Gln Pro Asn Leu Ile Pro Asn Gly Gly Gly Gln Met
 245 250 255
 Asn His Glu Ala Phe Ala Leu Ala His Gly Arg Pro Thr Tyr
 260 265 270

15

20

25

30

35

<210> 17
 <211> 882
 <212> DNA
 <213> Lotus japonicus

40

<400> 17
 atggctaatac cttggtggac aagccaggga gggttctctg gggttgaccc aggaacccat 60
 tcacctggct tgagcaaacg tcacacggac cttgtgatca atgaaaacag cagcgggtgg 120
 aatagagatg aagatgaaga tgataacagg gaagatgagc caaaagaagg tgcagttgag 180
 gttggaactc ggagaccaag ggaagacca ccgggatcca agaacaagcc aagaccaccc 240
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 ggagctgatg tcgcagaaag cgtggcccag tttgcgagga ggcgccagcg tggggtttgt 360
 gtgatgagcg ggagtggctc tgtggcaaac gttaccctga gacaacctgc ggctccgggt 420
 gctgtttag cactccatgg caggtttgag atcttatccc taactggggc gttcctacct 480
 ggccctgctc ctccaggatc cactggtcta acagtgtatc tttctggagg acagggtcag 540
 gtagtgggag ggagtgtggt ggggtctcta gttgcagcag gaccagttat ggtcattgct 600
 gcaacttttg ctaatgcaac atatgagagg ttgccacttg atgatgatga tgagggacct 660
 agtggggccg ctacggcggc aagcggagga ggaagtggat cgtctcctcc acctggaatt 720
 ggaattggca gtggtggggg tcatcaactg caggctggac tggttccaga tccatcatcc 780
 atgccgttgt ataatctgcc accaaatctg ttgtccaatg gaggaggagg acaagtgggg 840
 catgatgctc ttgcttgggc tcatggaaga acaccttact ga 882

55

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<210> 18
 <211> 293
 <212> PRT
 <213> Lotus japonicus

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<400> 18
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 Pro Gly Thr His Ser Pro Gly Leu Ser Lys Arg His Thr Asp Leu Val
 20 25 30
 Ile Asn Glu Asn Ser Ser Gly Gly Asn Arg Asp Glu Asp Glu Asp Asp
 35 40 45
 Asn Arg Glu Asp Glu Pro Lys Glu Gly Ala Val Glu Val Gly Thr Arg
 50 55 60
 Arg Pro Arg Gly Arg Pro Pro Gly Ser Lys Asn Lys Pro Arg Pro Pro
 65 70 75 80
 Ile Phe Val Thr Arg Asp Ser Pro Asn Ala Leu Arg Ser His Val Met
 85 90 95
 Glu Val Ala Gly Gly Ala Asp Val Ala Glu Ser Val Ala Gln Phe Ala
 100 105 110
 Arg Arg Arg Gln Arg Gly Val Cys Val Met Ser Gly Ser Gly Ser Val
 115 120 125
 Ala Asn Val Thr Leu Arg Gln Pro Ala Ala Pro Gly Ala Val Val Ala
 130 135 140
 Leu His Gly Arg Phe Glu Ile Leu Ser Leu Thr Gly Ala Phe Leu Pro
 145 150 155 160
 Gly Pro Ala Pro Pro Gly Ser Thr Gly Leu Thr Val Tyr Leu Ser Gly
 165 170 175
 Gly Gln Gly Gln Val Val Gly Gly Ser Val Val Gly Ser Leu Val Ala
 180 185 190
 Ala Gly Pro Val Met Val Ile Ala Ala Thr Phe Ala Asn Ala Thr Tyr
 195 200 205
 Glu Arg Leu Pro Leu Asp Asp Asp Asp Glu Gly Pro Ser Gly Ala Ala
 210 215 220
 Thr Ala Ala Ser Gly Gly Gly Ser Gly Ser Ser Pro Pro Pro Gly Ile
 225 230 235 240
 Gly Ile Gly Ser Gly Gly Gly His Gln Leu Gln Ala Gly Leu Val Pro
 245 250 255
 Asp Pro Ser Ser Met Pro Leu Tyr Asn Leu Pro Pro Asn Leu Leu Ser
 260 265 270
 Asn Gly Gly Gly Gly Gln Val Gly His Asp Ala Leu Ala Trp Ala His
 275 280 285
 Gly Arg Thr Pro Tyr
 290

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<210> 19
 <211> 708
 <212> DNA
 <213> Oryza sativa

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<400> 19
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 ccgcccgggt ccaagaacaa gcccaagccg cccatcttcg tgacgcggga cagcccgaac 180
 gcgctgcgca gccacgtcat ggaggtggcc ggcggcgccg atgtcgccga gtccatcgcg 240
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 gacgtggccc tgcgccagcc ggccgcgcgg agcgccgtgg tggcgctccg tgggcggttc 360
 gagatcctgt ccctgacggg gacgttcctg ccggggccgg cgccgccggg ctccaccggg 420
 ctgaccgtgt acctcgccgg cgggcagggg caggtggtgg gcggcagcgt ggtggggacg 480
 ctacccgcgg cggggccggg catggtgatc gcctccacct tcgccaacgc cacctacgag 540
 aggtgccgcg tggatcagga ggaggaggaa gcagcggcag gcggcatgat ggcgccggcg 600
 ccactcatgg ccggcgccgc cgatccacta cttttcgggc ggggaatgca cgacgcgggg 660
 cttgctgcat ggcacatgc ccgccctccg ccgcgcgcgc cctactag 708

55

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<210> 20
 <211> 235
 <212> PRT
 <213> Oryza sativa

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<400> 20
 Met Ala Ser Lys Glu Pro Ser Gly Asp His Asp His Glu Met Asn Gly
 1 5 10 15
 Thr Ser Ala Gly Gly Gly Glu Pro Lys Asp Gly Ala Val Val Thr Gly
 20 25 30
 Arg Asn Arg Arg Pro Arg Gly Arg Pro Pro Gly Ser Lys Asn Lys Pro
 35 40 45
 Lys Pro Pro Ile Phe Val Thr Arg Asp Ser Pro Asn Ala Leu Arg Ser
 50 55 60
 His Val Met Glu Val Ala Gly Gly Ala Asp Val Ala Glu Ser Ile Ala
 65 70 75 80
 His Phe Ala Arg Arg Arg Gln Arg Gly Val Cys Val Leu Ser Gly Ala
 85 90 95
 Gly Thr Val Thr Asp Val Ala Leu Arg Gln Pro Ala Ala Pro Ser Ala
 100 105 110
 Val Val Ala Leu Arg Gly Arg Phe Glu Ile Leu Ser Leu Thr Gly Thr
 115 120 125
 Phe Leu Pro Gly Pro Ala Pro Pro Gly Ser Thr Gly Leu Thr Val Tyr
 130 135 140
 Leu Ala Gly Gly Gln Gly Gln Val Val Gly Gly Ser Val Val Gly Thr
 145 150 155 160
 Leu Thr Ala Ala Gly Pro Val Met Val Ile Ala Ser Thr Phe Ala Asn
 165 170 175
 Ala Thr Tyr Glu Arg Leu Pro Leu Asp Gln Glu Glu Glu Glu Ala Ala
 180 185 190
 Ala Gly Gly Met Met Ala Pro Pro Pro Leu Met Ala Gly Ala Ala Asp
 195 200 205
 Pro Leu Leu Phe Gly Gly Gly Met His Asp Ala Gly Leu Ala Ala Trp
 210 215 220
 His His Ala Arg Pro Pro Pro Pro Pro Tyr
 225 230 235

20

25

30

35

<210> 21
 <211> 801
 <212> DNA
 <213> Oryza sativa

<400> 21
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 aaggagccgg aggcgagccc gacggggggc gcggcgccgg accacgccga cgagaacaac 120
 gaatccggcg gcggcgagcc gcgggagggc gccgtggtgg cggcgcccaa ccggcgcccc 180
 cgcgcccgcc cgccgggctc caagaacaag ccgaagccgc ccatcttcgt gacgcgcgac 240
 agccccaacg cgctgcgcag tcacgtcatg gaggtggccg gcggcgccga cgtcgccgac 300
 gccatcgcca agttctcgcg ccgcccagcag tcggcgcccg gcgtgctcag cggcgccggg 360
 acggtcgcca acgtcgcgct gcgcccagcc gcggcgcccg gcgcccgtcg cggcctgcac 420
 ggccgcttcg agatcctctc cctcaccggc accttcctcc caggcccggc gcctccgggt 480
 tccacggggc tcaccgtcta cctcgccggc ggccagggcc aggttgctcg cggcagcgtc 540
 gtggggtcgc tcatcgccgc gggcccggtc atggtgatcg cgtccacgtt cgccaacgcc 600
 acctacgagc gcctgccact ggaggaagaa gaggagggct caggcccggc catgcccggc 660
 ggcgcgcgag ccctcatggc cggcgccac gccatcgccg acccttcggc gctgccaatg 720
 ttcaacctgc cgccgagcaa cgggctcggc ggccggcgccg acggcttccc atggcgccgcg 780
 caccctgcc caccgtactg a 801

40

45

50

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<210> 22
 <211> 266
 <212> PRT
 <213> Oryza sativa

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<400> 22

Met Gly Leu Pro Glu Gln Pro Ser Gly Ser Ser Gly Pro Lys Ala Glu
 1 5 10 15
 Leu Pro Val Ala Lys Glu Pro Glu Ala Ser Pro Thr Gly Gly Ala Ala
 20 25 30
 Ala Asp His Ala Asp Glu Asn Asn Glu Ser Gly Gly Gly Pro Arg
 35 40 45
 Glu Gly Ala Val Val Ala Ala Pro Asn Arg Arg Pro Arg Gly Arg Pro
 50 55 60
 Pro Gly Ser Lys Asn Lys Pro Lys Pro Pro Ile Phe Val Thr Arg Asp
 65 70 75 80
 Ser Pro Asn Ala Leu Arg Ser His Val Met Glu Val Ala Gly Gly Ala
 85 90 95
 Asp Val Ala Asp Ala Ile Ala Gln Phe Ser Arg Arg Arg Gln Arg Gly
 100 105 110
 Val Cys Val Leu Ser Gly Ala Gly Thr Val Ala Asn Val Ala Leu Arg
 115 120 125
 Gln Pro Ser Ala Pro Gly Ala Val Val Ala Leu His Gly Arg Phe Glu
 130 135 140
 Ile Leu Ser Leu Thr Gly Thr Phe Leu Pro Gly Pro Ala Pro Pro Gly
 145 150 155 160
 Ser Thr Gly Leu Thr Val Tyr Leu Ala Gly Gly Gln Gly Gln Val Val
 165 170 175
 Gly Gly Ser Val Val Gly Ser Leu Ile Ala Ala Gly Pro Val Met Val
 180 185 190
 Ile Ala Ser Thr Phe Ala Asn Ala Thr Tyr Glu Arg Leu Pro Leu Glu
 195 200 205
 Glu Glu Glu Glu Gly Ser Gly Pro Pro Met Pro Gly Gly Ala Glu Pro
 210 215 220
 Leu Met Ala Gly Gly His Gly Ile Ala Asp Pro Ser Ala Leu Pro Met
 225 230 235 240
 Phe Asn Leu Pro Pro Ser Asn Gly Leu Gly Gly Gly Asp Gly Phe
 245 250 255
 Pro Trp Ala Ala His Pro Cys Pro Pro Tyr
 260 265

<210> 23

<211> 855

<212> DNA

<213> Populus tremuloides

<400> 23

atggcgaacc ggtggtggac agggcaagtg ggattgccgg ggatggacac atcaaccagt 60
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 gccaccgaga gtggtgctgg caaagaagat gagcaagaag acgaaagaga aaatagcgac 180
 gagcctagag aaggcgctat agatatcgcc tctcgccgcc ctagaggccg tccaccaggg 240
 tccaagaaca agcctaagcc accaattttc gttactcgag acagccctaa tgcactcaag 300
 agtcatgtga tggagatagc tagtggatct gatatagctg aaaatttagc ttgttttgca 360
 aggaagagac aaagaggagt ttgtgtgctt agtggaaagt gtatggtaac caatgtaacc 420
 ctcaagcaac cttctgcctc aggtgctgtt atggctctcc atggtaggtt tgagattttg 480
 tcaactcactg gagcgttctt gcctggacca gccccacctg gagcgacagg actaactata 540
 tatttagccg gagggcaagg acaagtggta ggaggcagtg tggtaggatc actagttgca 600
 tcaggaccgg taatggttat tgctgcaaca ttttcaaata ctacttatga gagattgcca 660
 ctagaagatg aagaggaagg cagtgggtgg gcacaagggc agctcggtgg cggcaacggg 720
 agcgggtgagg gtaatggtgg gggcatgggg gatccagcaa catcaatgcc agtttatcaa 780
 ttgccaaata tgggtgcctaa tggacaattg aaccatgaag gatatgggtg ggctcacggc 840
 agaccaccct attag 855

<210> 24

<211> 284

<212> PRT

<213> Populus tremuloides

<400> 24

Met Ala Asn Arg Trp Trp Thr Gly Gln Val Gly Leu Pro Gly Met Asp
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 Thr Ser Thr Ser Ser Ser Pro Met Lys Lys Pro Asp Leu Gly Ile
 20 25 30
 Ser Met Ser Asn Asn Asn Arg Glu Ala Thr Glu Ser Gly Ala Gly Lys
 35 40 45
 Glu Asp Glu Gln Glu Asp Glu Arg Glu Asn Ser Asp Glu Pro Arg Glu
 50 55 60
 Gly Ala Ile Asp Ile Ala Ser Arg Arg Pro Arg Gly Arg Pro Pro Gly
 65 70 75 80
 Ser Lys Asn Lys Pro Lys Pro Pro Ile Phe Val Thr Arg Asp Ser Pro
 85 90 95
 Asn Ala Leu Lys Ser His Val Met Glu Ile Ala Ser Gly Ser Asp Ile
 100 105 110
 Ala Glu Asn Leu Ala Cys Phe Ala Arg Lys Arg Gln Arg Gly Val Cys
 115 120 125
 Val Leu Ser Gly Ser Gly Met Val Thr Asn Val Thr Leu Lys Gln Pro
 130 135 140
 Ser Ala Ser Gly Ala Val Met Ala Leu His Gly Arg Phe Glu Ile Leu
 145 150 155 160
 Ser Leu Thr Gly Ala Phe Leu Pro Gly Pro Ala Pro Pro Gly Ala Thr
 165 170 175
 Gly Leu Thr Ile Tyr Leu Ala Gly Gly Gln Gly Gln Val Val Gly Gly
 180 185 190
 Ser Val Val Gly Ser Leu Val Ala Ser Gly Pro Val Met Val Ile Ala
 195 200 205
 Ala Thr Phe Ser Asn Ala Thr Tyr Glu Arg Leu Pro Leu Glu Asp Glu
 210 215 220
 Glu Glu Gly Ser Gly Gly Ala Gln Gly Gln Leu Gly Gly Gly Asn Gly
 225 230 235 240
 Ser Gly Glu Gly Asn Gly Gly Gly Met Gly Asp Pro Ala Thr Ser Met
 245 250 255
 Pro Val Tyr Gln Leu Pro Asn Met Val Pro Asn Gly Gln Leu Asn His
 260 265 270
 Glu Gly Tyr Gly Trp Ala His Gly Arg Pro Pro Tyr
 275 280

<210> 25

<211> 885

<212> DNA

<213> Solanum tuberosum

<400> 25

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 gcgggttcgc cttctctcaa gaagccagat ctaggcgtat caatgaacga tatagtgggt 120
 ggtagtggtg gtcgatgaga agatagggac catagcgacg accctaaaga ggggtgcagtc 180
 gaagtagcca ctgctcgacc cagaggtcga ccagctggct caaagaacaa acctaaacca 240
 ccaatatttg ttacaaggga tagccctaac gcacttagaa gccacgtaat ggaagttgct 300
 aatggagctg atgtggcgga aagtatagct caatttgcta ggaaaagaca aagaggtgtt 360
 tgtgttttga gtgctactgg aactgttact aatgtaacc taagacaacc atctgctcct 420
 ggagctgtca tggcattaca cggccggttc gagatcttat cgttgaccgg agctttctta 480
 cctggaccgg cccctcctgg atcaacaggg ttgactatat acctagcagg aggacaagga 540
 caagttgtgg gaggaagtgt agtagggtct ttagtggtct ccggaccagt tatggtaatt 600
 gcatcaactt tttttaatgc aacatatgag aggctacct tggaggagga ggaagaaggc 660
 ggtggaacgg tggcccaagg acaacttggt ggtggtggat cgccaccggg aatgggagga 720
 agtggtggtg gtggtggagg acaacaacaa caaggtggtg gtggtatggg tgatattcca 780
 tcatcaaata tgccagtata taatttgcca ccaaatttgc taccaaatgg tggacaaatg 840
 aacctgaag catttggttg ggcacatgga cgccctcctt ttttaa 885

<210> 26

<211> 294

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<212> PRT

<213> Solanum tuberosum

<400> 26

5 Met Ser Asn Pro Trp Trp Thr Gly Gln Val Gly Leu Gln Gly Val Glu
1 5 10 15
Thr Ser Ser Ser Ala Gly Ser Pro Ser Leu Lys Lys Pro Asp Leu Gly
20 25 30
Val Ser Met Asn Asp Ile Val Gly Gly Ser Gly Ser His Asp Glu Asp
35 40 45
10 Arg Asp His Ser Asp Asp Pro Lys Glu Gly Ala Val Glu Val Ala Thr
50 55 60
Arg Arg Pro Arg Gly Arg Pro Ala Gly Ser Lys Asn Lys Pro Lys Pro
65 70 75 80
Pro Ile Phe Val Thr Arg Asp Ser Pro Asn Ala Leu Arg Ser His Val
85 90 95
15 Met Glu Val Ala Asn Gly Ala Asp Val Ala Glu Ser Ile Ala Gln Phe
100 105 110
Ala Arg Lys Arg Gln Arg Gly Val Cys Val Leu Ser Ala Thr Gly Thr
115 120 125
20 Val Thr Asn Val Thr Leu Arg Gln Pro Ser Ala Pro Gly Ala Val Met
130 135 140
Ala Leu His Gly Arg Phe Glu Ile Leu Ser Leu Thr Gly Ala Phe Leu
145 150 155 160
Pro Gly Pro Ala Pro Pro Gly Ser Thr Gly Leu Thr Ile Tyr Leu Ala
165 170 175
25 Gly Gly Gln Gly Gln Val Val Gly Gly Ser Val Val Gly Ser Leu Val
180 185 190
Ala Ser Gly Pro Val Met Val Ile Ala Ser Thr Phe Phe Asn Ala Thr
195 200 205
Tyr Glu Arg Leu Pro Leu Glu Glu Glu Glu Glu Gly Gly Gly Thr Val
210 215 220
30 Ala Gln Gly Gln Leu Gly Gly Gly Gly Ser Pro Pro Gly Met Gly Gly
225 230 235 240
Ser Gly Gly Gly Gly Gly Gly Gln Gln Gln Gln Gly Gly Gly Gly Met
245 250 255
Gly Asp Ile Pro Ser Ser Asn Met Pro Val Tyr Asn Leu Pro Pro Asn
260 265 270
35 Leu Leu Pro Asn Gly Gly Gln Met Asn His Glu Ala Phe Gly Trp Ala
275 280 285
His Gly Arg Pro Pro Phe
290

<210> 27

<211> 939

<212> DNA

<213> Thlaspi caerulescens

<400> 27

45 atggcggaatc catggtggac aggacaagtg aatctctccg gccttgaaac gacgccgcct 60
ggttcctctc agttaagaa atcagatctc cacatctcca tgaacatggc catggactca 120
ggtcataaca accatcatca tcaccaagaa gtcgacaaca ataacaacaa cgatgacgac 180
agagataact tgagcggcga tgaacacgag ccacgtgaag gagccgtaga agccccacg 240
cgccgtccac gtggacgtcc tgctggttcc aagaacaaac caaagccacc gatctttgtc 300
acgcgcgatt ctccaaacgc tctcaagagc catgtcatgg agatcgctag tgggactgac 360
50 gtcatcgaaa ccctagctac tttcgctagg cggcgccaac gtggcatctg catcttgagc 420
ggcaacggca cgggtggctaa cgtcactctc cgccaaccat catctgccgc agttgctgcg 480
gctcccgggg gtgcggcggg tttggcttta caagggagggt ttgagattct ctctttaaca 540
ggatcgttct tgcctggacc tgcctcacct ggatccaccg gtttaacat ctacttagcc 600
ggtggtcaag gtcaggtcgt tggaggaagt gtggtggggc cattgatggc ggctgggtccg 660
gttatgttaa tcgcggccac gttttctaag gcgacttacg agagattgcc tttggaggag 720
55 gaagaggcgg ctgagagagg cgggtggagga ggcagcgtcc caggacaact cggaggggga 780
ggctcgccgc tgagtagcgg tgggtgtgga ggggatggca atcaaggact tccggtgtac 840

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aatatgcccg gaaatcttgt ttctaattgt ggcggaggcg gaggacagat gagtggccaa 900
 gaagcttatg gttgggctca agctaggtca ggatttttaa 939

<210> 28
 <211> 312
 <212> PRT
 <213> *Thlaspi caerulescens*

<400> 28

Met Ala Asn Pro Trp Trp Thr Gly Gln Val Asn Leu Ser Gly Leu Glu
 1 5 10 15
 Thr Thr Pro Pro Gly Ser Ser Gln Leu Lys Lys Ser Asp Leu His Ile
 20 25 30
 Ser Met Asn Met Ala Met Asp Ser Gly His Asn Asn His His His His
 35 40 45
 Gln Glu Val Asp Asn Asn Asn Asn Asn Asp Asp Asp Arg Asp Asn Leu
 50 55 60
 Ser Gly Asp Glu His Glu Pro Arg Glu Gly Ala Val Glu Ala Pro Thr
 65 70 75 80
 Arg Arg Pro Arg Gly Arg Pro Ala Gly Ser Lys Asn Lys Pro Lys Pro
 85 90 95
 Pro Ile Phe Val Thr Arg Asp Ser Pro Asn Ala Leu Lys Ser His Val
 100 105 110
 Met Glu Ile Ala Ser Gly Thr Asp Val Ile Glu Thr Leu Ala Thr Phe
 115 120 125
 Ala Arg Arg Arg Gln Arg Gly Ile Cys Ile Leu Ser Gly Asn Gly Thr
 130 135 140
 Val Ala Asn Val Thr Leu Arg Gln Pro Ser Ser Ala Ala Val Ala Ala
 145 150 155 160
 Ala Pro Gly Gly Ala Ala Val Leu Ala Leu Gln Gly Arg Phe Glu Ile
 165 170 175
 Leu Ser Leu Thr Gly Ser Phe Leu Pro Gly Pro Ala Pro Pro Gly Ser
 180 185 190
 Thr Gly Leu Thr Ile Tyr Leu Ala Gly Gly Gln Gly Gln Val Val Gly
 195 200 205
 Gly Ser Val Val Gly Pro Leu Met Ala Ala Gly Pro Val Met Leu Ile
 210 215 220
 Ala Ala Thr Phe Ser Asn Ala Thr Tyr Glu Arg Leu Pro Leu Glu Glu
 225 230 235 240
 Glu Glu Ala Ala Glu Arg Gly Gly Gly Gly Gly Ser Val Pro Gly Gln
 245 250 255
 Leu Gly Gly Gly Gly Ser Pro Leu Ser Ser Gly Gly Gly Gly Gly Asp
 260 265 270
 Gly Asn Gln Gly Leu Pro Val Tyr Asn Met Pro Gly Asn Leu Val Ser
 275 280 285
 Asn Gly Gly Gly Gly Gly Gly Gln Met Ser Gly Gln Glu Ala Tyr Gly
 290 295 300
 Trp Ala Gln Ala Arg Ser Gly Phe
 305 310

<210> 29
 <211> 876
 <212> DNA
 <213> *Vitis vinifera*

<400> 29

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 tcacctgcaa tgaagaaacc agatctggga atatccatga atgaaaatgg aggaagcggg 120
 agcggaggcg gaggagagga agaagaggaa aaagaaaaca gtgatgagcc cagagagggg 180
 gcaattgagg tggctacgcg caggcctagg ggcggccgc ctggctccaa gaacaagcca 240
 aaacctccga tttttgtgac aaggacagc cctaaccgtc tgcgcagcca cgttatggag 300
 gtggcaaaacg gctccgacat cacagaaagc atagcccaat tcgcgagaag gcggcaacga 360
 ggcgtctgcg tgctcagcgc aagtgggaca gtcataaacg taacgcttcg ccagccttct 420

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gccctggtg gtgcagttat ggcacttcat ggccgattcg aaattccttc cttaccgggc 480
 gcgttcctac cgggaccagc gccaccagc tccactggac taaccatata cctagcaggc 540
 ggtcaagctc aggtcgtggg tggtagcgtg gtgggttcac tcatagcggc aggtccagtt 600
 atggtgattg cagctacctt ttcgaatgca acctacgaga ggctccccct agaagacgaa 660
 5 gaagaggcgg gcagcgcagc acaggagcag ctgctggcg gcggaggcgg tggtgggtca 720
 ccgccagggg ttggcggcag tggggggcag cagcaggcag ggatggcaga tccttcctcc 780
 atgccggttt ataatttgcc accaaatttg cttccaaatg gtggacaact gaaccatgat 840
 gcttatggtt gggcacatgg gcgccagcct tactag 876

 <210> 30
 10 <211> 291
 <212> PRT
 <213> Vitis vinifera

 <400> 30
 15 Met Ala Asn Arg Trp Trp Ala Gly Gln Val Gly Leu Gln Gly Val Asp
 1 5 10 15
 Thr Ser Ser Ala Ser Pro Ala Met Lys Lys Pro Asp Leu Gly Ile Ser
 20 25 30
 Met Asn Glu Asn Gly Gly Ser Gly Ser Gly Gly Gly Glu Glu Glu
 35 40 45
 20 Glu Glu Lys Glu Asn Ser Asp Glu Pro Arg Glu Gly Ala Ile Glu Val
 50 55 60
 Ala Thr Arg Arg Pro Arg Gly Arg Pro Pro Gly Ser Lys Asn Lys Pro
 65 70 75 80
 Lys Pro Pro Ile Phe Val Thr Arg Asp Ser Pro Asn Ala Leu Arg Ser
 85 90 95
 25 His Val Met Glu Val Ala Asn Gly Ser Asp Ile Thr Glu Ser Ile Ala
 100 105 110
 Gln Phe Ala Arg Arg Arg Gln Arg Gly Val Cys Val Leu Ser Ala Ser
 115 120 125
 Gly Thr Val Met Asn Val Thr Leu Arg Gln Pro Ser Ala Pro Gly Gly
 130 135 140
 30 Ala Val Met Ala Leu His Gly Arg Phe Glu Ile Leu Ser Leu Thr Gly
 145 150 155 160
 Ala Phe Leu Pro Gly Pro Ala Pro Pro Gly Ser Thr Gly Leu Thr Ile
 165 170 175
 Tyr Leu Ala Gly Gly Gln Ala Gln Val Val Gly Gly Ser Val Val Gly
 180 185 190
 35 Ser Leu Ile Ala Ala Gly Pro Val Met Val Ile Ala Ala Thr Phe Ser
 195 200 205
 Asn Ala Thr Tyr Glu Arg Leu Pro Leu Glu Asp Glu Glu Glu Ala Gly
 210 215 220
 Ser Ala Ala Gln Glu Gln Leu Ala Gly Gly Gly Gly Gly Gly Ser
 225 230 235 240
 Pro Pro Gly Ile Gly Gly Ser Gly Gly Gln Gln Gln Ala Gly Met Ala
 245 250 255
 Asp Pro Ser Ser Met Pro Val Tyr Asn Leu Pro Pro Asn Leu Leu Pro
 260 265 270
 45 Asn Gly Gly Gln Leu Asn His Asp Ala Tyr Gly Trp Ala His Gly Arg
 275 280 285
 Gln Pro Tyr
 290

 <210> 31
 50 <211> 783
 <212> DNA
 <213> Vitis vinifera

 <400> 31
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 gataaccccg gcacaggaga cgatgaagaa gagaaagaca acgaaggcga gccacaggag 120
 ggtgcagtag aagtcggcac tcgtagacca agaggtcgcc cgcctggatc caaaaacaag 180

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5
 10
 15
 20
 25
 30
 35
 40
 45

cccaaacc	ctattttcgt	cacgcgcgac	agcccgaacg	cccttcggag	ccacgtgatg	240
gaggtggccg	gcggccacga	cggtgccgaa	agcgtcgccc	agttcgcccc	taggcgtcaa	300
cgaggggtct	gcgtcctcag	cggcagcggc	tccgtagcca	acgtgactct	gagacagccc	360
gccgcgcctg	gcgccgtggt	ggcactccat	ggaagattcg	agattctgtc	cctaacagga	420
gcattcctcc	ccggacctgc	ccctcccggc	tccactggac	tcaccgtgta	cctcgccgga	480
ggtcagggcc	aggttggtgg	aggaagtgtg	gttggtatcac	tggtagcggc	aggcccgggtg	540
atagtgatag	ccgccacttt	tgcgaacgca	acatacgaaa	gactgcctct	ggaagaagaa	600
gaagaaggtg	ggcaggcgcc	gccgcggagt	ggttcgccgc	ctgcaattgg	aagcagtggt	660
ggacagcatc	actctggcct	gccggagctg	cccatataca	atctgccacc	gaacctactc	720
cctaaccggcg	gcccaattgag	tcatgacccc	tactcatggg	ctcatgctcg	gcccccttac	780
tga						783

<210> 32
 <211> 260
 <212> PRT
 <213> Vitis vinifera

<400> 32
 Met Asp Pro Ala Ala Val Ser Pro Met Leu Asn Lys Arg Asp Arg Glu
 1 5 10 15
 Ile Ser Ile Asn Asp Asn Pro Gly Thr Gly Asp Asp Glu Glu Lys
 20 20 25 30
 Asp Asn Glu Gly Glu Pro Thr Glu Gly Ala Val Glu Val Gly Thr Arg
 35 40 45
 Arg Pro Arg Gly Arg Pro Pro Gly Ser Lys Asn Lys Pro Lys Pro Pro
 50 55 60
 Ile Phe Val Thr Arg Asp Ser Pro Asn Ala Leu Arg Ser His Val Met
 65 70 75 80
 Glu Val Ala Gly Gly His Asp Val Ala Glu Ser Val Ala Gln Phe Ala
 85 90 95
 Arg Arg Arg Gln Arg Gly Val Cys Val Leu Ser Gly Ser Gly Ser Val
 100 105 110
 Ala Asn Val Thr Leu Arg Gln Pro Ala Ala Pro Gly Ala Val Val Ala
 115 120 125
 Leu His Gly Arg Phe Glu Ile Leu Ser Leu Thr Gly Ala Phe Leu Pro
 130 135 140
 Gly Pro Ala Pro Pro Gly Ser Thr Gly Leu Thr Val Tyr Leu Ala Gly
 145 150 155 160
 Gly Gln Gly Gln Val Val Gly Gly Ser Val Val Gly Ser Leu Val Ala
 165 170 175
 Ala Gly Pro Val Ile Val Ile Ala Ala Thr Phe Ala Asn Ala Thr Tyr
 180 185 190
 Glu Arg Leu Pro Leu Glu Glu Glu Gly Gly Gln Ala Pro Pro
 195 200 205
 Pro Ser Gly Ser Pro Pro Ala Ile Gly Ser Ser Gly Gly Gln His His
 210 215 220
 Ser Gly Leu Pro Glu Leu Pro Ile Tyr Asn Leu Pro Pro Asn Leu Leu
 225 230 235 240
 Pro Asn Gly Gly Gln Leu Ser His Asp Pro Tyr Ser Trp Ala His Ala
 245 250 255
 Arg Pro Pro Tyr
 260

<210> 33
 <211> 810
 <212> DNA
 <213> Zea mays

<400> 33
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 gaccgggaga acggcgccac gggcgagccc aaggaaggcg cgggtggtggc gggcaaccgg 120
 cgcccccgcg ggcgcccgcc ggggtccaag aacaagccca agccgcccac cttcgtgacg 180
 cgcgacagcc ccaacgcgct gcgcagccac gtgatggagg tggccggcgg cgccgacgtg 240

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gccgagtcca tcgcccactt cgcgcgccgc aggcagcgcg gcgtgtgcgt gctcagcggc 300
 gcgggcaccg tcgccgacgt ggcgctccgc cagcccgcgg ctccggggcg cgtgggtcgcc 360
 ctccgcggcc gcttcgagat cctctcgctc accggcacgt tcctgccggg ccccgcgccg 420
 cggggctcca cggggctcac cgtgtacctc gcgggcggcc aggggcaggt cgtcggcggc 480
 5 agcgtcgctcg gcacgctcac cgcggcgggg cccgtcatgg tgatggcgtc cacgttcgcc 540
 aacgccacct acgagaggct gccgctggac gacgccgacg aggagcccgc cgggcagcag 600
 gcggcgcagc tgccctcccg accgggcgga gggcagccta tggtaatggg cgggatggcc 660
 gaccctcag cgggtgccaat gttcggcggc gccggcgggtg tgccgccaag cctcatgcca 720
 gcaggggccg cagccgcctc ctccggtgcg ggcctgcagc tcgggcacga ccgacttgca 780
 10 tgggtcatg cacggccacc gccatactag 810

<210> 34
 <211> 269
 <212> PRT
 <213> Zea mays

15 <400> 34
 Met Ala Pro Ser Ser Lys Asp Gly Ala Thr Ala Thr Glu Gln Pro Thr
 1 5 10 15
 Ser Gly Asp Asp Arg Glu Asn Gly Thr Gly Glu Pro Lys Glu
 20 20 25 30
 Gly Ala Val Val Ala Gly Asn Arg Arg Pro Arg Gly Arg Pro Pro Gly
 35 40 45
 Ser Lys Asn Lys Pro Lys Pro Pro Ile Phe Val Thr Arg Asp Ser Pro
 50 55 60
 Asn Ala Leu Arg Ser His Val Met Glu Val Ala Gly Gly Ala Asp Val
 65 70 75 80
 25 Ala Glu Ser Ile Ala His Phe Ala Arg Arg Gln Arg Gly Val Cys
 85 90 95
 Val Leu Ser Gly Ala Gly Thr Val Ala Asp Val Ala Leu Arg Gln Pro
 100 105 110
 Ala Ala Pro Gly Ala Val Val Ala Leu Arg Gly Arg Phe Glu Ile Leu
 115 120 125
 30 Ser Leu Thr Gly Thr Phe Leu Pro Gly Pro Ala Pro Pro Gly Ser Thr
 130 135 140
 Gly Leu Thr Val Tyr Leu Ala Gly Gly Gln Gly Gln Val Val Gly Gly
 145 150 155 160
 Ser Val Val Gly Thr Leu Thr Ala Ala Gly Pro Val Met Val Met Ala
 165 170 175
 35 Ser Thr Phe Ala Asn Ala Thr Tyr Glu Arg Leu Pro Leu Asp Asp Ala
 180 185 190
 Asp Glu Glu Pro Ala Gly Gln Gln Ala Ala Gln Leu Pro Pro Gly Pro
 195 200 205
 Gly Gly Gly Gln Pro Met Val Met Gly Gly Met Ala Asp Pro Ser Ala
 210 215 220
 40 Val Pro Met Phe Gly Gly Ala Gly Gly Val Pro Pro Ser Leu Met Pro
 225 230 235 240
 Ala Gly Ala Ala Ala Ala Ser Ser Gly Ala Gly Leu Gln Leu Gly His
 245 250 255
 45 Asp Arg Leu Ala Trp Ala His Ala Arg Pro Pro Pro Tyr
 260 265

<210> 35
 <211> 2194
 <212> DNA
 50 <213> Oryza sativa

<400> 35
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 aaatataaaa tgagacctta tatatgtagc gctgataact agaactatgc aagaaaaact 120
 catccaccta ctttagtggc aatcgggcta aataaaaaag agtcgctaca ctagtctcgt 180
 55 tttccttagt aattaagtgg gaaaatgaaa tcattattgc ttagaatata cgttcacatc 240
 tctgtcatga agttaaatga ttcgaggtag ccataattgt catcaaactc ttcttgaata 300

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aaaaaatctt tctagctgaa ctcaatgggt aaagagagag atttttttta aaaaaataga 360
 atgaagatat tctgaacgta ttggcaaaaga tttaaacata taattatata atttttatagt 420
 ttgtgcattc gtcatatcgc acatcattaa ggacatgtct tactccatcc caatttttat 480
 ttagtaatta aagacaattg acttattttt attattttatc ttttttcgat tagatgcaag 540
 5 gtacttacgc acacactttg tgcctcatgtg catgtgtgag tgcacctcct caatacacgt 600
 tcaactagca acacatctct aatatactct gcctatttaa tacatttagg tagcaatatc 660
 tgaattcaag cactccacca tcaccagacc actttttaata atatctaaaa tacaaaaaat 720
 aatttttacag aatagcatga aaagtatgaa acgaactatt taggtttttc acatacaaaa 780
 aaaaaaagaa ttttgctcgt gcgcgagcgc caatctccca tattgggcac acaggcaaca 840
 10 acagagtggc tgcccacaga acaaccacaa aaaaacgatg atctaacgga ggacagcaag 900
 tccgcaacaa ccttttaaca gcaggctttg cggccaggag agaggaggag aggcaaaaga 960
 aaccaagcat cctccttctc ccactataaa attcctcccc ccttttcccc tctctatata 1020
 ggaggcatcc aagccaagaa gagggagaca accaaggaca cgcgactagc agaagccgag 1080
 cgaccgcctt ctcgatccat atcttccggt cgagtctctg ttcgatctct tccctcctcc 1140
 acctcctcct cacagggtat gtgcctccct tcggttggtc ttggatttat tgttctaggt 1200
 15 tgtgtagtagc gggcgttgat gttaggaaag gggatctgta tctgtgatga ttctgtttct 1260
 tggatttggg atagaggggt tcttgatgtt gcatgttatc gggtcgggtt gattagtagt 1320
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 gtaataaagt acggttggtt ggtcctcgat tctggtagtg atgcttctcg atttgacgaa 1500
 gctatccttt gtttattccc tattgaacaa aaataatcca actttgaaga cggccccgtt 1560
 20 gatgagattg aatgattgat tcttaagcct gtccaaaatt tcgcagctgg cttgtttaga 1620
 tacagtagtc cccatcacga aattcatgga aacagttata atcctcagga acaggggatt 1680
 ccctgttctt ccgatttgct ttagtcccag aatttttttt cccaaatatc ttaaaaagtc 1740
 actttctggt tcagttcaat gaattgattg ctacaaataa tgcttttata gcgttatcct 1800
 agctgtagtt cagttaatag gtaatacccc tatagtttag tcaggagaag aacttatccg 1860
 25 atttctgatc tccattttta attatatgaa atgaactgta gcataagcag tattcatttg 1920
 gattattttt tttatttagct ctcaccctt cattattctg agctgaaagt ctggcatgaa 1980
 ctgtcctcaa ttttgttttc aaattcacat cgattatcta tgcattatcc tcttgtatct 2040
 acctgtagaa gtttcttttt ggttattcct tgactgcttg attacagaaa gaaatttatg 2100
 aagctgtaat cgggatagtt atactgcttg ttcttatgat tcatttcctt tgtgcagttc 2160
 30 ttggtgtagc ttgccacttt caccagcaaa gttc 2194

 <210> 36
 <211> 173
 <212> PRT
 <213> Artificial sequence

 35 <220>
 <223> conserved domain comprised in SEQ ID NO: 2

 <400> 36
 Glu Pro Arg Glu Gly Ala Val Glu Ala Pro Thr Arg Arg Pro Arg Gly
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 40 Arg Pro Ala Gly Ser Lys Asn Lys Pro Lys Pro Pro Ile Phe Val Thr
 20 25 30
 Arg Asp Ser Pro Asn Ala Leu Lys Ser His Val Met Glu Ile Ala Ser
 35 40 45
 Gly Thr Asp Val Ile Glu Thr Leu Ala Thr Phe Ala Arg Arg Arg Gln
 50 55 60
 45 Arg Gly Ile Cys Ile Leu Ser Gly Asn Gly Thr Val Ala Asn Val Thr
 65 70 75 80
 Leu Arg Gln Pro Ser Thr Ala Ala Val Ala Ala Ala Pro Gly Gly Ala
 85 90 95
 50 Ala Val Leu Ala Leu Gln Gly Arg Phe Glu Ile Leu Ser Leu Thr Gly
 100 105 110
 Ser Phe Leu Pro Gly Pro Ala Pro Pro Gly Ser Thr Gly Leu Thr Ile
 115 120 125
 Tyr Leu Ala Gly Gly Gln Gly Gln Val Val Gly Gly Ser Val Val Gly
 130 135 140
 55 Pro Leu Met Ala Ala Gly Pro Val Met Leu Ile Ala Ala Thr Phe Ser
 145 150 155 160
 Asn Ala Thr Tyr Glu Arg Leu Pro Leu Glu Glu Glu Glu

165

170

5 <210> 37
 <211> 14
 <212> PRT
 <213> Artificial sequence

 <220>
 <223> AT hook

 10 <220>
 <221> VARIANT
 <222> (8)..(8)
 <223> /replace ="Ala"

 15 <220>
 <221> VARIANT
 <222> (11)..(11)
 <223> /replace ="Arg"

 20 <400> 37
 Arg Arg Pro Arg Gly Arg Pro Pro Gly Ser Lys Asn Lys Pro
 1 5 10

 25 <210> 38
 <211> 125
 <212> PRT
 <213> Artificial sequence

 <220>
 <223> PPC domain (DUF296) comprised in SEQ ID NO: 2

 30 <400> 38
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 1 5 10 15
 Thr Leu Ala Thr Phe Ala Arg Arg Arg Gln Arg Gly Ile Cys Ile Leu
 20 25 30
 Ser Gly Asn Gly Thr Val Ala Asn Val Thr Leu Arg Gln Pro Ser Thr
 35 35 40 45
 Ala Ala Val Ala Ala Ala Pro Gly Gly Ala Ala Val Leu Ala Leu Gln
 50 55 60
 Gly Arg Phe Glu Ile Leu Ser Leu Thr Gly Ser Phe Leu Pro Gly Pro
 65 70 75 80
 40 Ala Pro Pro Gly Ser Thr Gly Leu Thr Ile Tyr Leu Ala Gly Gly Gln
 85 90 95
 Gly Gln Val Val Gly Gly Ser Val Val Gly Pro Leu Met Ala Ala Gly
 100 105 110
 Pro Val Met Leu Ile Ala Ala Thr Phe Ser Asn Ala Thr
 115 120 125

 45 <210> 39
 <211> 52
 <212> DNA
 <213> Artificial sequence

 50 <220>
 <223> primer: prm8135

 <400> 39
 ggggacaagt ttgtacaaaa aagcaggctt aaacaatggc gaatccatgg tg
 55
 <210> 40
 <211> 50

52

<212> DNA
<213> Artificial sequence

<220>
<223> primer: prm8136

<400> 40
ggggaccact ttgtacaaga aagctgggtt aaaaaccatt ttaacgcacg

50

<210> 41
<211> 948
<212> DNA
<213> Brassica oleracea

<400> 41
atgcgaaatc catggtggac aggacaagtg aatctctcca gtctcgaaac gacgccgccg 60
agttcctctc agttaagac accagatctc cacatctcca tgaacatggc catggtctca 120
ggtcataaca accaccatca tcatcaccaa gaagtcaaca ccaacaacaa caacgaagac 180
gataagagaca acttgagcgg cgacgaccgc gagccacgtg aaggagccgt ggaagctccc 240
acgcgccgac cacgtggacg tcctgctggt tccaagaaca aaccaaagcc accaatcttt 300
gtcacgcgtg attctccaaa cgctctcaag agccatgtca tggagatcgc tagtgggact 360
gatgtcatag aaaccctagc tactttcgct agggcgccgc aacgtggcat ctgcactttg 420
agcggtaacg gcacggtggc taacgtcaca ctccgtcaac catcagtggc tcccgttgca 480
gctgcccctg gtgggtgcggc tgtattggcg ttacaaggga ggtttgagat tctttctcta 540
accggttctt tcttacctgg accggtccca cctggatcca ctggtttaac tatttactta 600
gctgggtggc aagggtcaggt tgttggagga agcgtgggtg gggcattgat ggctgctggt 660
ccggtgatgc taatcgctgc cacgttttct aatgcgactt atgagagatt acctttggat 720
gaggaagaag cggctgaaaag aggtggcggt ggaagcgacg gaggagtggg tccagggcag 780
ctcgggggcg taggttcccc gctgagtagt ggtggcggtg gaggccacgg gaaccaagga 840
cttcccgcacg ataatatgcc cggaaacctt gcttctaata gcggtggagg aggacagatg 900
agcagccaag aagcgtacgg ttgggctcaa gctaggtcag gatttttaa 948

<210> 42
<211> 315
<212> PRT
<213> Brassica oleracea

<400> 42

Met	Arg	Asn	Pro	Trp	Trp	Thr	Gly	Gln	Val	Asn	Leu	Ser	Ser	Leu	Glu
1				5					10					15	
Thr	Thr	Pro	Pro	Ser	Ser	Ser	Gln	Leu	Lys	Thr	Pro	Asp	Leu	His	Ile
			20					25					30		
Ser	Met	Asn	Met	Ala	Met	Val	Ser	Gly	His	Asn	Asn	His	His	His	His
		35				40						45			
His	Gln	Glu	Val	Asn	Thr	Asn	Asn	Asn	Asn	Glu	Asp	Asp	Arg	Asp	Asn
		50				55				60					
Leu	Ser	Gly	Asp	Asp	Arg	Glu	Pro	Arg	Glu	Gly	Ala	Val	Glu	Ala	Pro
65					70				75					80	
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35 40 45
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Gly Ala Ile Glu Thr Asn Thr Ser Thr Arg Arg Pro Arg Gly Arg Pro
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85 90 95

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 35 40 45
 55 Met Lys Asn Gln Tyr Glu Ala Ser Gly Glu Ser Asn Asn Ala Glu Asn
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75

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Lys

<210> 47

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<212> DNA

<213> *Oryza sativa*

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10 <213> Chlamydomonas reinhardtii

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	tccattgctc	ttcatgggtg	agctcttgct	ccgtattatc	ttaatgaatc	aacaggctgg	600
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 cagatccttg ccagcctcgt catgaatcca ccaaaggctg gagatgcac atagtcttca 1080
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 35 40 45
 Ile Leu Tyr Cys Asn Ile Gly Asn Pro Gln Ser Leu Gly Gln Lys Pro
 50 55 60
 Val Thr Phe Phe Arg Glu Val Ile Ala Leu Cys Asp His Pro Cys Leu
 25 65 70 75 80
 Leu Glu Lys Glu Glu Thr Lys Ser Leu Phe Ser Ala Asp Ala Ile Ser
 85 90 95
 Arg Ala Thr Thr Ile Leu Ala Ser Ile Pro Gly Arg Ala Thr Gly Ala
 100 105 110
 Tyr Ser His Ser Gln Gly Ile Lys Gly Leu Arg Asp Ala Ile Ala Ala
 30 115 120 125
 Gly Ile Ala Ser Arg Asp Gly Tyr Pro Ala Asn Ala Asp Asp Ile Phe
 130 135 140
 Leu Thr Asp Gly Ala Ser Pro Gly Val His Met Met Met Gln Leu Leu
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 35 Ile Arg Asn Glu Lys Asp Gly Ile Leu Cys Pro Ile Pro Gln Tyr Pro
 165 170 175
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 180 185 190
 Tyr Leu Asn Glu Ser Thr Gly Trp Gly Leu Glu Ile Ser Asp Leu Lys
 195 200 205
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 Asn Gln Arg Asp Ile Val Lys Phe Cys Lys Asn Glu Gly Leu Val Leu
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 45 Leu Ala Asp Glu Val Tyr Gln Glu Asn Ile Tyr Val Asp Asn Lys Lys
 260 265 270
 Phe Asn Ser Phe Lys Lys Ile Ala Arg Ser Met Gly Tyr Asn Glu Asp
 275 280 285
 Asp Leu Pro Leu Val Ser Phe Gln Ser Val Ser Lys Gly Tyr Tyr Gly
 290 295 300
 50 Glu Cys Gly Lys Arg Gly Gly Tyr Met Glu Ile Thr Gly Phe Ser Ala
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 Pro Val Arg Glu Gln Ile Tyr Lys Val Ala Ser Val Asn Leu Cys Ser
 325 330 335
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 Ala Gly Asp Ala Ser Tyr Ala Ser Tyr Lys Ala Glu Lys Asp Gly Ile

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[illegible]

tataactctg gcattttttt tattgtaggg caatatgttt tccattattt tccattaaaa 2460
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10 <220>
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25

Claims

- 30 1. A method for enhancing yield-related traits in plants grown under abiotic stress conditions relative to control plants, comprising increasing expression in a plant of a nucleic acid sequence encoding a GRP polypeptide, wherein said GRP polypeptide is a metallothionein 2a (MT2a) polypeptide as represented by SEQ ID NO:46 or an orthologue, paralogue, or homologue thereof, and optionally selecting for plants grown under abiotic stress conditions having enhanced yield-related traits.
- 35 2. A method according to claim 1, wherein said GRP polypeptide as represented by SEQ ID NO:46 and an orthologue, paralogue, or homologue thereof, have an InterPro entry IPR000347, described as plant metallothionein, family 15.
- 40 3. Method according to claims 1 or 2, wherein said GRP polypeptide has in increasing order of preference at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% or more amino acid sequence identity to the GRP polypeptide as represented by SEQ ID NO:46.
- 45 4. Method according to any preceding claim 1 to 3, wherein said nucleic acid sequence encoding a GRP polypeptide is represented by the nucleic acid sequence of SEQ ID NO:45 or a portion thereof, or a sequence capable of hybridising with the nucleic acid sequence of SEQ ID NO:45 or a portion thereof.
5. Method according to any preceding claim 1 to 4 wherein said increased expression is effected by introducing and expressing in a plant a nucleic acid sequence encoding said GRP polypeptide.
- 50 6. Method according to any preceding claim 1 to 5, wherein said abiotic stress is an osmotic stress, selected from one or more of the following: water stress, salt stress, oxidative stress and ionic stress.
7. Method according to claim 6, wherein said water stress is drought stress.
8. Method according to claim 6, wherein said ionic stress is salt stress.
- 55 9. Method according to any preceding claim 1 to 8, wherein said enhanced yield-related traits are one or more of: increased aboveground biomass, increased total seed yield per plant, increased number of filled seeds, increased total number of filled seeds, increased number of primary panicles and increased seed fill rate, relative to control

plants.

10. Method according to any preceding claim 1 to 9, wherein said nucleic acid sequence is operably linked to a constitutive promoter, preferably to a GOS2 promoter, most preferably to a GOS2 promoter from rice.

11. Method according to any preceding claim 1 to 10, wherein said nucleic acid sequence encoding a GRP polypeptide is of plant origin, preferably from a dicotyledonous plant, further preferably from the family Brassicaceae, more preferably from *Arabidopsis thaliana*.

12. Use of a nucleic acid sequence encoding a GRP polypeptide in enhancing yield-related traits in plants grown under abiotic stress conditions.

13. Use of a nucleic acid sequence encoding a GRP polypeptide according to claim 12, wherein said enhanced yield-related traits are selected from one or more of: increased aboveground biomass, increased total seed yield per plant, increased number of filled seeds, increased total number of filled seeds, increased number of primary panicles and increased seed fill rate, relative to control plants.

14. Use of a nucleic acid sequence encoding a GRP polypeptide according to claim 13, wherein said abiotic stress is an osmotic stress, selected from one or more of the following: water stress, salt stress, oxidative stress and ionic stress.

15. Use of a nucleic acid sequence encoding a GRP polypeptide according to claim 14, said water stress is drought stress and/or said ionic stress is salt stress.

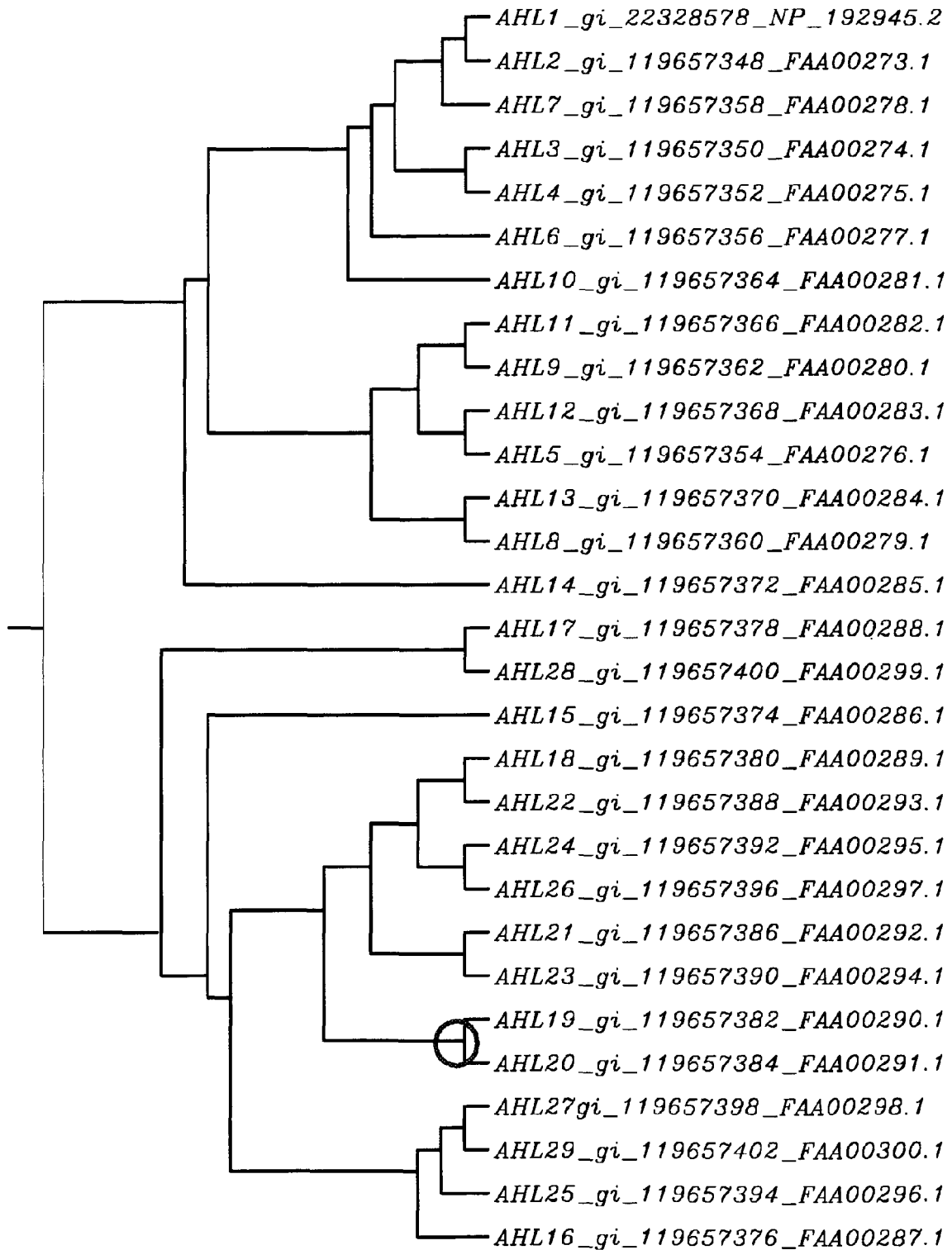


FIGURE 1

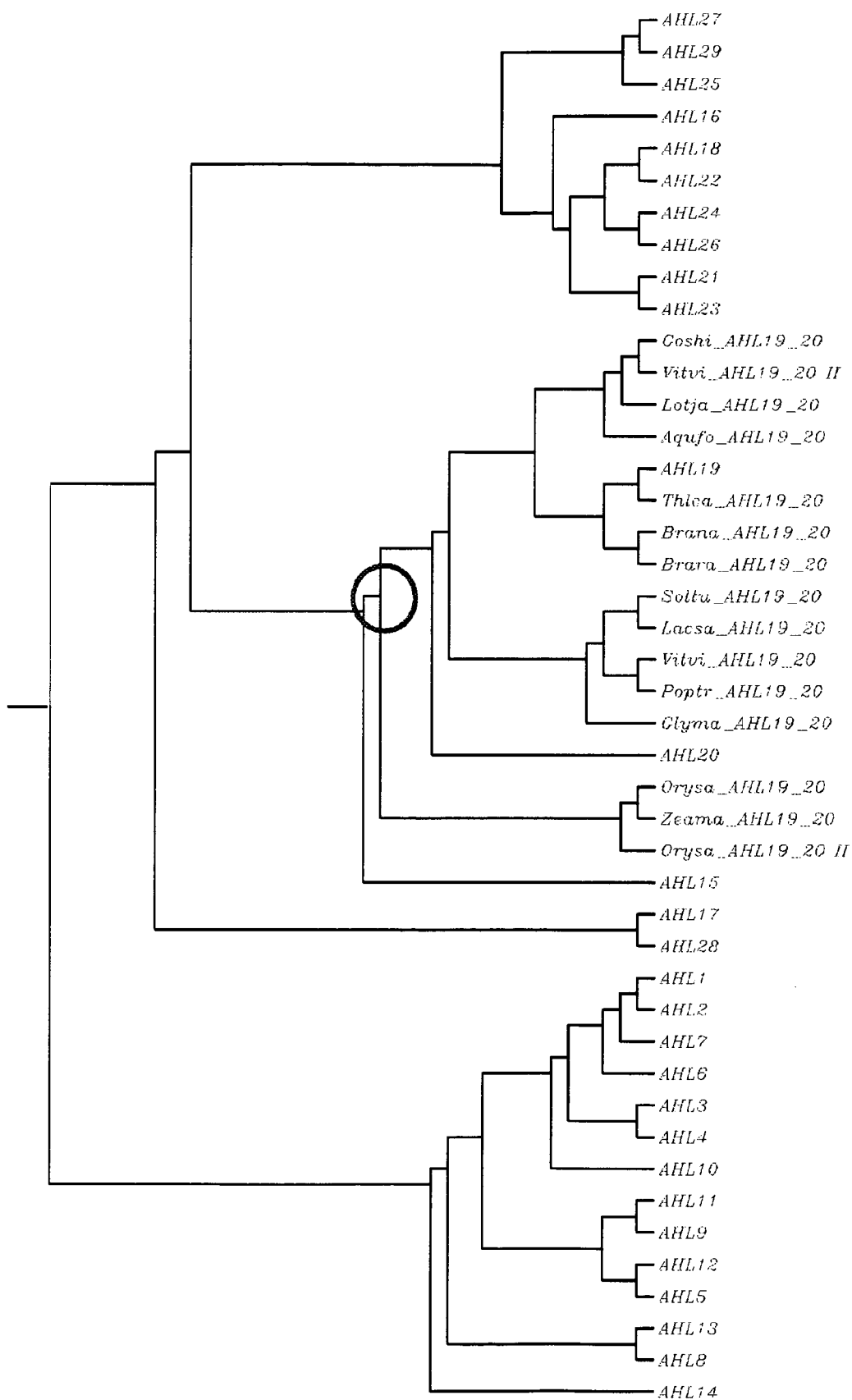


FIGURE 2

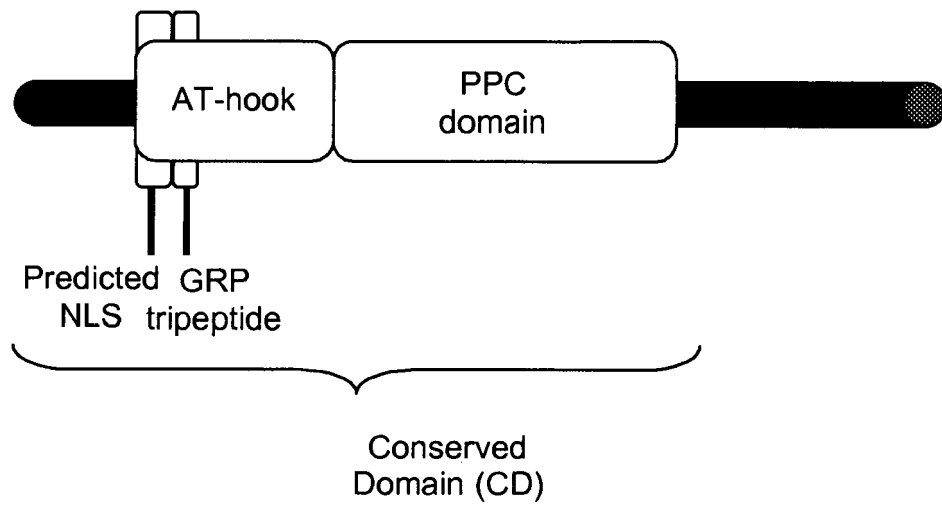


FIGURE 3

CLUSTAL W (1.83) multiple sequence alignment of the Conserved Domain (CD)

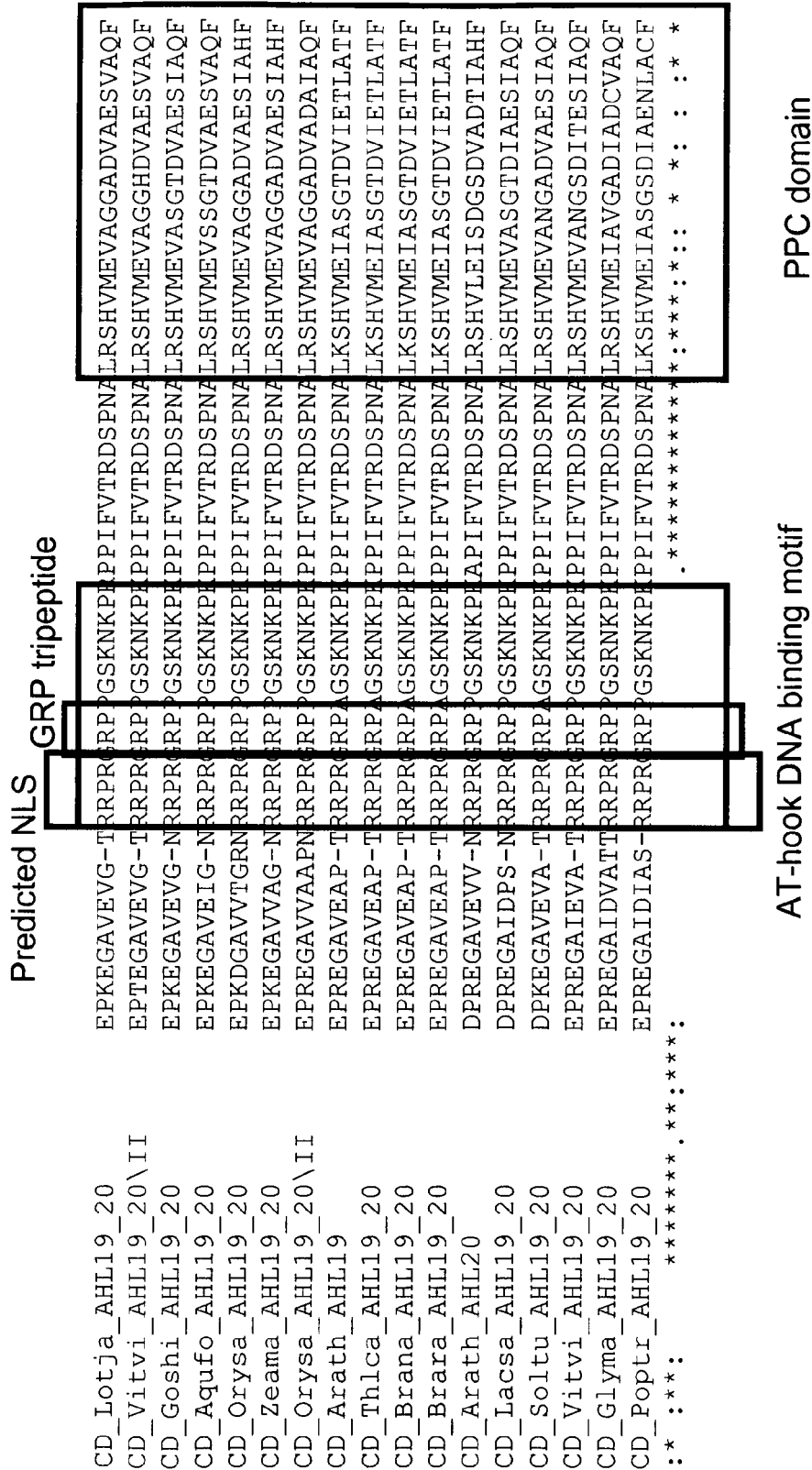


FIGURE 4

[illegible]

PPC domain (continued)

FIGURE 4 (continued)

CLUSTAL W (1.83) multiple sequence alignment of the **Conserved Domain** (CD) (cont'd)

CD_Lotja_AHL19_20	PPGSTGLTVYLSGGQGVVGGSVVGGSLVAAAGPVMVIAATFANAT	VERLPLDDDD
CD_Vitvi_AHL19_20\II	PPGSTGLTVYLAGGQGVVGGSVVGGSLVAAAGPVIVIAATFANAT	VERLPLEEEEE
CD_Goshi_AHL19_20	PPGSTGLTVYLAGGQGVVGGSVVGGSLIAAGPVMVIAATFSNAT	VERLPLEDEEE
CD_Aqufo_AHL19_20	PPGSTGLTVYLAGGQGVVGGSVVGGTLLIAAGPVIVIAATFANAT	VERLPIEEEE
CD_Orysa_AHL19_20	PPGSTGLTVYLAGGQGVVGGSVVGGTLLTAAGPVMVIASTFANAT	VERLPLDQEE
CD_Zeama_AHL19_20	PPGSTGLTVYLAGGQGVVGGSVVGGTLLTAAGPVMVMASTFANAT	VERLPLDDAD
CD_Orysa_AHL19_20\II	PPGSTGLTVYLAGGQGVVGGSVVGGSLIAAGPVMVIASTFANAT	VERLPLEEEEE
CD_Arath_AHL19	PPGSTGLTVYLAGGQGVVGGSVVGGPLMAAGPVMVIAATFSNAT	VERLPLEEEEE
CD_Thlca_AHL19_20	PPGSTGLTVYLAGGQGVVGGSVVGGPLMAAGPVMVIAATFSNAT	VERLPLEEEEE
CD_Brana_AHL19_20	PPGSTGLTVYLAGGQGVVGGSVVGGPLMAAGPVMVIAATFSNAT	VERLPLDEEE
CD_Brara_AHL19_20	PPGSTGLTVYLAGGQGVVGGSVVGGPLMAAGPVMVIAATFSNAT	VERLPLDEEE
CD_Arath_AHL20	PPGSTGLTVYLAGVQGVVGGSVVGGPLLAIGSVMVIAATFSNAT	VERLPMEEEE
CD_Lacsa_AHL19_20	PPGSTGLTVYLAGGQGVVGGSVVGGSLVASGPVMVIAATFSNAT	VERLPVEEE-
CD_Soltu_AHL19_20	PPGSTGLTVYLAGGQGVVGGSVVGGSLVASGPVMVIASTFFNAT	VERLPLEEEEE
CD_Vitvi_AHL19_20	PPGSTGLTVYLAGGQAQGVVGGSVVGGSLIAAGPVMVIAATFSNAT	VERLPLEDEEE
CD_Glyma_AHL19_20	PPGATGLTVYLAGGQGVVGGSVVGGVGPLVAAGPVLVMAATFSNAT	VERLPLEDDDD
CD_Poptr_AHL19_20	PPGATGLTVYLAGGQGVVGGSVVGGSLVASGPVMVIAATFSNAT	VERLPLEDEEE
:*:***:*.*.****.***	* * * .*.****.***	*****:

PPC domain (continued)

FIGURE 4 (continued)

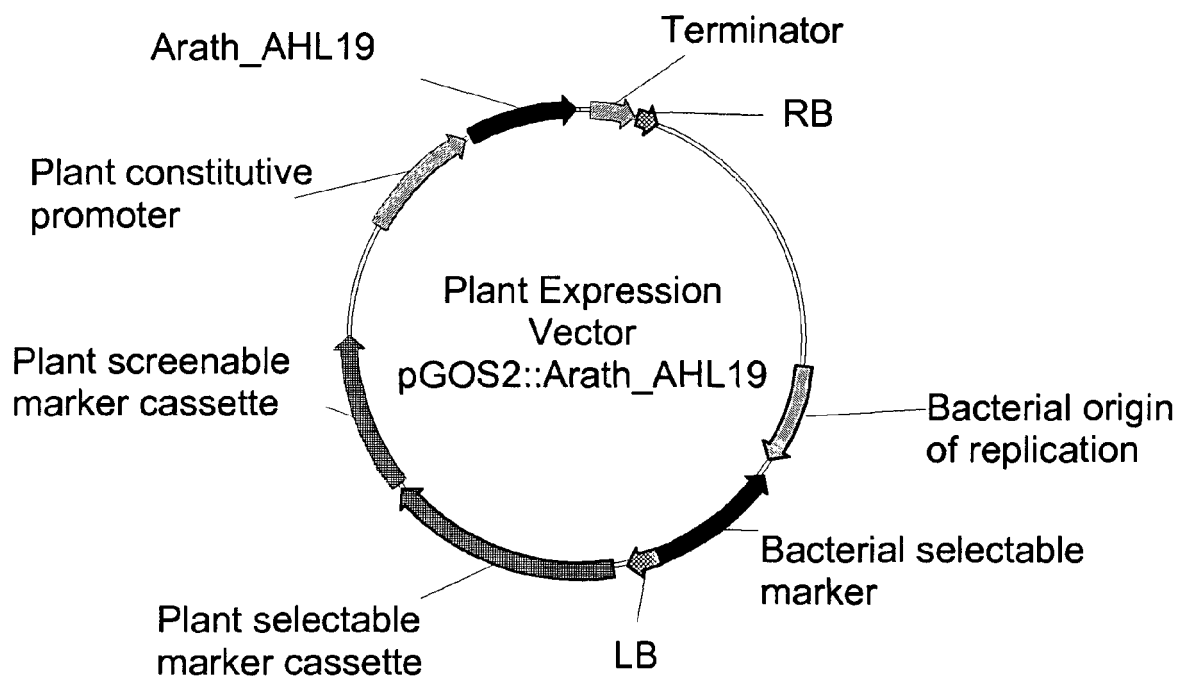


FIGURE 5

SEQ ID NO : 1 Arabidopsis thaliana AHL19 nucleic acid sequence AT3G04570 NP_566232

ATGGCGAATCCATGGTGGACAGGACAAGTGAACCTATCCGGCCTCGAAAACGACGCCGCTGGTTCC
TCTCAGTTAAAGAAACCAGATCTCCACATCTCCATGAACATGGCCATGGACTCAGGTCACAATAAT
CATCACCATCACCAAGAAGTCGATAACAACAACAACGACGACGATAGAGACAACCTTGAGTGGAGAC
GACCACGAGCCACGTGAAGGAGCCGTAGAAGCCCCACGCGCCGTCCACGTGGACGTCCTGCTGGT
TCCAAGAACAACCAAGCCACCGATCTTCGTCACCTCGGATTCTCCAAATGCTCTCAAGAGCCAT
GTCATGGAGATCGCTAGTGGGACTGACGTCATCGAAACCTAGCTACTTTTGCTAGGCGGCGTCAA
CGTGGCATCTGCATCTTGAGCGGAAATGGCACAGTGGCTAACGTCACCTCCGTCAACCCTCGACC
GCTGCCGTTGCGGCGGCTCCTGGTGGTGGGCTGTTTTGGCTTTACAAGGGAGGTTGAGATTCTT
TCTTTAACCGGTTCTTTCTTGCCAGGACCGGCTCCACCTGGTTCCACCGGTTTAACGATTTACTTA
GCCGGTGGTCAAGGTCAGGTTGTTGGAGGAAGCGTGGTGGGCCCATTTGATGGCAGCAGGTCCGGTG
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GCAGAGAGAGGCGGTGGTGGAGGCAGCGGAGGAGTGGTTCCGGGGCAGCTCGGAGGCGGAGGTTTCG
CCACTAAGCAGCGGTGCTGGTGGAGGCGACGGTAACCAAGGACTTCCGGTGTATAATATGCCGGGA
AATCTTGTCTTAATGGTGGCAGTGGTGGAGGAGGACAGATGAGCGGCCAAGAAGCTTATGGTTGG
GCTCAAGCTAGGTCAGGATTTTAA

SEQ ID NO: 2 Arabidopsis thaliana AHL19 translated polypeptide sequence AT3G04570

MANPWWTGQVNLSGLETTTPPGSSQLKKPDLHISMNMAMDSGHNHNNHHHQEVDNNNNDDDRDNLSGD
DHEPREGAVEAPTRRPRGRPAGSKNPKPPIFVTRDSPNALKSHVMEIASGTDVIETLATFARRRQ
RGICILSGNGTVANVTLRQPSTAAVAAAPGGA AVLALQGRFEILSLTGSFLPGPAPPGSTGLTIYL
AGGQGGVVGGSVVGPLMAAGPVMLIAATFSNATYERLPLEEEEAERGGGGSGGVVPGQLGGGGS
PLSSGAGGGDGNQGLPVYNMPPGNLVSNGGSGGGGQMSGQEA YGWAQARS GF

SEQ ID NO: 3 Arabidopsis thaliana AHL20 nucleic acid sequence AT4G14465 NM_117526.3

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GGCCATCACCAAAACCATCACCAACCAAGTCTTCTTACCAAGGAGATCTTGGAAATAGCCATGAAT
CAGAGCCAAGACAACGACCAAGACGAAGAAGATGATCCTAGAGAAGGAGCCGTTGAGGTGGTCAAC
CGTAGACCAAGAGGTAGACCACCAGGATCCAAAAACAAACCCAAAGCTCCAATCTTTGTGACAAGA
GACAGCCCCAACGCACTCCGTAGCCATGTCTTGAGATCTCCGACGGCAGTGACGTCGCCGACACA
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AACGTCACCTCCGCCAAGCCGCCGACCAGGAGGTGTGGTCTCTCTCCAAGGCAGGTTTGAAATC
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TTAGCCGGGGTCCAGGGTCAGGTCGTTGGAGGTAGCGTTGTAGGCCCACTCTTAGCCATAGGGTCG
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GACGGTGGCGGCTCAAGACAGATTCACGGAGGCGGTGACTCACCGCCAGAATCGGTAGTAACCTG
CCTGATCTATCAGGGATGGCCGGGCCAGGCTACAATATGCCGCCGATCTGATTCCAAATGGGGCT
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SEQ ID NO: 4 Arabidopsis thaliana AHL20 translated polypeptide sequence AT4G14465

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RRRPRGRPAGSKNPKAPIFVTRDSPNALRSHVLEISDGSDVADTIAHFSRRRQRGVCVLSGTGSA
NVTLRQAAAPGGVVS LQGRFEILSLTGAF LPGPSPPGSTGLTVYLAGVQGGVVGSPVLLAIGS
VMVIAATFSNATYERLPMEEEDGGGSRQIHGGGDSPPRIGSNLPDLSGMAGPGYNMPPHLIPNGA
GQLGHEPYTWVHARPPY

FIGURE 6

SEQ ID NO: 5 *Aquilegia formosa* x *Aquilegia pubescens* AHL19/20 nucleic acid sequence contig of DT758489, DT758488.1

ATGGCAAATCCATGGTGGACTGGGCAGGTGGGACTGCCTGGTGGTTTAGAAACAGGAGCGGGTTCA
CCTGCGTTTAGAAAACGCGATCGAGATTTATCGATGAATGAAAGTGTAAGTGGTGGTAGAGGAGGT
GAGGATGACGATGAAAGAGATAACGGTGATGAGCCTAAAGAAGGTGCGGTAGAGATAGGTAACCGC
CGTCCAAGGGGCCGACCACCTGGGTCAAAGAACAAGCCAAAACACCGATTTTTGTGACTCGCGAT
AGCCCAAACGCGCTTAGGAGCCATGTGATGGAGGTCTCAAGTGGGACTGATGTAGCCGAAAGTGTA
GCCCAATTTGCTAGGAGGCGACAAAGAGGTGTTTGTGTACTTAGTGGTAGTGGCGTAGTGGCCAAT
GTAACATTGCGACAACCTTCAGCTCCAAGTGCAGTTGTGGCTCTGCAAGGTCGATTTCGAAATATTG
TCTCTAACTGGTTTCACTTTCCTGGCTGGGCGGCACCCCCAGGATCAACTGGGCTGACGGTCTACTTG
GCAGGCGGTGAGGGGCAAGTGGTAGGCGGTAGCGTGGTGGTACTCTTATTGCAGCTGGTCCAGTT
ATTGTGATTGCAGCAACATTTGCAAATGCAACATATGAGAGACTACCAATTGAGGAGGAGGAGGAT
GCAGGAAGTGGAGGTGAGGGACAACCTCCAGGGCGGTGCAGGAAGCTCACCACCACCAATTGGAAGC
AGTACCGGGCAACAGCAACCAGGGATGCCAGACCTATCCTCTTTGCCAGTGTATAATATGCCACCA
AACCTACTCCAAAATGGAGGGCAGATGAACCAGCAAGAAGCATATGCTTGGGCTCATGCTCGGCCA
CGTATTGA

SEQ ID NO: 6 *Aquilegia formosa* x *Aquilegia pubescens* AHL19/20 translated polypeptide sequence

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RPRGRPPGSKNPKPKPIFVTRDSPNALRSHVMEVSSGTDVAESVAQFARRRQRGVCVLSGSGVVAN
VTLRQPSAPSAVVALQGRFEILSLTGSFLPGPAPPGSTGLTVYLAGGQGVVGGSVVGTLIAAGPV
IVIAATFANATYERLPIEEEEEDAGSGGQQLQGAGSSPPPIGSSTGQQQPGMPDLSSLPVYNMPP
NLLQNGGQMNQOEAYAWAHARPPY

SEQ ID NO: 7 *Brassica napus* AHL19/20 nucleic acid sequence CS226287

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CACCACCATCATCACCAGAAGTCAACACCAACAACAACGAAGACGATAGAGACAACCTGAGC
GGCGACGACCACGAGCCACGTGAAGGAGCCGTGGAAGCTCCACGCGCCGACCACGTGGACGTCTCT
GCTGGTTCCAAGAACAACCAAGCCACCAATCTTTGTACGCGTGACTCTCCAAACGCTCTCAAG
AGCCATGTGATGGAGATCGCTAGTGGGACTGACGTCATCGAAACCCTAGCTACTTTTCGCTAGGCGG
CGCCAACGTGGCATCTGCATCTTGAGCGGTAACGGCACGGTGGCTAACGTCACACTCCGTCAACCA
TCAGTGGCTCCCGTTGCAGCTGCCCCTGGTGGTGGCGCTGTATTGGCGTTACAAGGGAGGTTTGAG
ATTCTTTCTCTAACCGGTTCTTTCTTACCTGGACCGGCTCCACCTGGATCCACTGGTTTAACTATT
TACTTAGCTGGTGGTCAAGGTCAGGTTGTTGGAGGAAGCGTGGTGGGGCCATTGATGGCTGCTGGT
CCGGTGATGCTAATCGCTGCCACGTTTCTAATGCGACTTATGAGAGATTACCTTTGGATGAGGAA
GAAGCGGCTGAAAGAGGTGGCGGTGGAAGCGACGGAGGAGTGGTTCAGGGCAGCTCGGGGGCGTA
GGTTCCCCGCTGAGTAGTGGTGGCGGTGGAGGCCATGGGAACCAAGGACTTCCCGCGTATAATATG
CCCGGAAATCTTGCTTCTAATGGCGGTGGAGGAGGACAGATGAGCGGCCAAGAAGCTTACGGTTGG
GCTCAAGCTAGGTCAGGATTTTAA

SEQ ID NO: 8 *Brassica napus* AHL19/20 translated polypeptide sequence

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YLAGGQGVVGGSVVGPLMAAGPVMLIAATFSNATYERLPLDEEEEAERGGGGSDGGVVPGLGGV
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FIGURE 6 (continued)

SEQ ID NO: 9 Brassica rapa AHL19/20 nucleic acid sequence AC189468

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 AACAAACGAAGACGATAGAGACAACCTTGAGCGGCGACGACCACGAGCCACGTGAAGGAGCCGTGGAA
 GCTCCACGCGCCGACCACGTGGACGTCCTGCTGGTTCCAAGAACAACCAAGCCACCAATCTTT
 GTCACGCGTGACTCTCCAAACGCTCTCAAGAGCCATGTCATGGAGATCGCTAGTGGGACTGACGTC
 ATCGAAACCCTAGCTACTTTTCGCTAGGCGGCGCCAACGTGGCATCTGCATCTTGAGCGGTAACGGC
 ACGGTGGCTAACGTCACACTCCGTCAACCATCAGTGGCTCCCGTTGCAGCTGCCCCCTGGTGGTGCG
 GCTGTATTGGCGTTACAAGGGAGGTTTGAGATTCTTTCTCTAACCGGTTCTTTCTTACCTGGACCG
 GCTCCACCTGGATCCACTGGTTTAACTATTTACTTAGCTGGTGGTCAAGGTCAGGTTGTTGGAGGA
 AGCGTGGTGGGGCCATTGATGGCTGCTGGTCCGGTGATGCTAATCGCTGCCACGTTTTCTAATGCG
 ACTTATGAGAGATTACCTTTGGATGAGGAAGAAGCGGCTGAAAGAGGTGGCGGTGGAAGCGACGGA
 GGAGTGGTTCCAGGGCAGCTCGGGGGCGTAGGTTCCCCGCTGAGTAGTGGTGGCGGTGGAGGCCAT
 GGGAAACCAAGGACTTCCCGCGTATAATATGCCCGGAAATCTTGCTTCTAATGGCGGTGGAGGAGGA
 CAGATGAGCGGCCAAGAAGCTTACGGTTGGGCTCAAGCTAGGTCAGGATTTTAA

SEQ ID NO: 10 Brassica rapa AHL19/20 translated polypeptide sequence

MANPWWTGQVNLSGLETPPSSSQLKTPDLHISMNMAMDSGHNNHHHHHHQEVNTNNNNEDDRDNL
 GDDHEPREGAVEAPTRRPRGRPAGSKNPKPPIFVTRDSPNALKSHVMEIASGTDVIETLATFARR
 RQRGICILSNGTIVANVTLRQPSVAPVAAAPGGA AVLALQGRFEILSLTGSFLPGPAPPGSTGLTI
 YLAGGQGGVVGGSVVGPLMAAGPVMLIAATFSNATYERLPLDEEEAAERGGGSDGGVVPGLGGV
 GSPLSSGGGGGHGNQGLPAYNMPGNLASNGGGGGQMSGQEAYGWAQARSGF

SEQ ID NO: 11 Glycine max AHL19/20 nucleic acid sequence CS137412

ATGGCCAACCGGTGGTGGACCGGTCGGTGGGTCTAGAGAACTCTGGCCACTCGATGAAAAAACCG
 GATCTGGGGTTTTTCCATGAACGAGAGTACGGTGACGGGGAACCATATAGGAGAAGAAGATGAGGAC
 AGAGAAAACAGCGACGAGCCAAGAGAGGGAGCTATTGACGTCGCCACCACGCGCCGCCCTAGGGGA
 CGTCCACCGGGCTCCAGAAACAAGCCGAAACCGCCGATATTGCTCACCCGAGACAGCCCTAACGCG
 CTGCGGAGCCACGTCATGGAGATTGCCGTGCGGAGCCGACATCGCCGACTGCGTGGCGCAGTTCGCT
 CGGAGGCGCCAGCGCGGGGTTTTCCATTCTCAGCGGCAGCGGGACCGTCGTCAACGTCAATCTCCGG
 CAACCCACGGCACCCGGCGCCGTCATGGCGCTCCACGGCCGCTTCGACATCCTCTCCCTCACCGGC
 TCCTTTCTCCCTGGGCCGTCCCCCTCCCGGCGCCACCGGGCTCACAACTACCTCGCCGGAGGCCAG
 GGGCAGATCGTCGGCGGCGGAGTGGTGGGCCCCGCTCGTGGCGGCGGGCCCCGTATTGGTAATGGCG
 GCTACTTTTTTCCAATGCTACGTATGAAAGATTGCCTTTAGAGGATGATGATCAGGAACAACACGGC
 GGCGGAGGCGGAGGAGGTTCCCGCAGGAAAAAACCGGGGGTCCCGGCGAGGCGTCGTCGTCGATT
 TCGGTTTATAACAATAATGTTCCCTCCGAGTTTAGGTCTTCCGAATGGGCAACATCTGAACCATGAA
 GCTTATTCTTCTCCTTGGGGTCATTCTCCTCATGCCAGACCTCCTTTCTAA

SEQ ID NO: 12 Glycine max AHL19/20 translated polypeptide sequence

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 RPPGSRNPKPPIFVTRDSPNALRSHVMEIAVGADIADCVAQFARRRQRGVSILSGSGTVVNVNLR
 QPTAPGAVMALHGRFDILSLTGSFLPGPSPPGATGLTIYLAGGQGGIVGGGVVGPLVAAGPVLVMA
 ATFSNATYERLPLEDDDQEQHGGGGGGGSPQEKTTGGPGEASSISVYNNNVPPSLGLPNGQHLNHE
 AYSSPWGHSHPARPPF

FIGURE 6 (continued)

SEQ ID NO: 13 *Gossypium hirsutum* AHL19/20 nucleic acid sequence DW519458

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AACAAAAGTAGAAGCAACGGAAGAGGGGATGATGATGATGAAGATAGAGACACCGGCGATGAGCCT
AAAGAAGGAGCGGTCGAGGTCGGTAACCGAAGACCCGAGGTCGTCCACCGGGATCCAAAAACAAG
CCTAAACCACCCATTTTTGTGACAAGGGATAGCCCTAACGCGCTCCGTAGTCATGTTATGGAAGTC
GCAAGTGGAAACCGATGTAGCCGAGAGTATAGCCCAATTCGCTCGGAGAAGACAACGTGGAGTTTGT
TTGCTTAGCGGCAGCGGCTCGGTCGCCAACGTTACTCTAAGACAACCGGCAGCACCCGGCGCGGTG
GTTGCCCTTCATGGAAGGTTTGAAATTTTGTCTTTGACCGGGGCTTTTCTCCCGGACCGGCTCCA
CCGGGATCGACAGGGCTCACCGTGTACTTAGCTGGTGGTCAAGGACAAGTTGTTGGAGGAAGTGTT
GTCGGCTCACTTATAGCAGCAGGGCCTGTTATGGTCATTGCAGCAACTTTTCCAACGCAACTTAT
GAAAGACTGCCTTTAGAAGATGAAGAAGAAGTTGTAAGCGCCGGTCACGGTGGACCGATGCAAGGC
GGAGCAAACGATTCACCGCCGAAATTGGGAGTAGCGGAGGCGGCGGTTACACACAGGTCTGCCT
GATCCATCTTCACTTCCAATATACAATTTGCCTCCTAATTTACTCTCAAATGGAGGGCAACTAGGG
CATGAACCCTATGGTTGGACACATGGGAGACCACCCTATTAA

SEQ ID NO: 14 *Gossypium hirsutum* AHL19/20 translated polypeptide sequence

MDPAGNSPALNKRDL EISMNDANKSRSNRGDDDDDEDRDTGDEPKEGAVEVGNRRPRGRPPGSKNK
PKPPIFVTRDSPNALRSHVMEVASGTDVAESIAQFARRRQRGVCLLSGSGSVANVTLRQPAAPGAV
VALHGRFEILSLTGAFLPGPAPPGSTGLTVYLAGGQGVVGGSVVGSLIAAGPVMVIAATFSNATY
ERLPLEDEEEVVSAGHGGPMQGGANDSPPEIGSSGGGSHTGLPDPSSLPIYNLPPNLLSNGGQLG
HEPYGWITHGRPPY

SEQ ID NO: 15 *Lactuca sativa* AHL19/20 nucleic acid sequence DW047323

ATGTCTAACCGATGGTGGACCGGCCAGGTCAACGTGGCAGGCGTAGAAACATCATCTCAGGCGATC
AAGAAACCAGATCTGGGTATCTCAATGAATGATACCACCACAGGAAGTGAAGAAGATGAAAGAGAC
AACAAACAGCGATGATCCAAGAGAAGGTGCAATTGACCCTTCTAACCGTAGGCCACGAGGCCGACCT
CCGGGATCCAAAAACAAACAAAGCCACCGATTTTCGTACCCAGAGACAGCCCTAACGCCCTCCGC
AGCCACGTCATGGAGGTAGCGAGTGGTACAGATATCGCAGAAAGTATAGCTCAATTCAGCCGAAAA
CGACAACGCGGTGTGTGTGTGATGAGTGCTAGCGGCACAGTCATGAATGTAACCCTAAGACAACCT
TCGGCACCTGGCTCAGTCATGGCTCTACAAGGCCGGTTCGAGATTTTATCCCTAACCGGTGCCTTC
TTACCGGGTCCTTCTCCTCCTGGATCCACCGGGCTCACTATATATTTAGCTGGTGGCCAGGGCCAG
GTTGTGGGCGGTAGCGTGGTGGGATCATTGGTGGCATCAGGACCAGTGATGGTTATAGCAGCCACG
TTCTCCAACGCCACATATGAAAGACTCCCGGTTGAGGAAGAGGAGGAAGCAGATACCGTGACACCT
GGGCTAGGTGGTGGTGGATCACCACCGCAACTCGGAATGGGTGATCAGAATCCGATGGCAGGGTAT
AATATGCAGCCGAATTTGATCCCGAATGGTGGTGGACAGATGAACCATGAAGCTTTTGCTTTGGCT
CATGGCCGGCCACGTACTAG

SEQ ID NO: 16 *Lactuca sativa* AHL19/20 translated polypeptide sequence

MSNRWWTGQVNVAGVETSSQAIKKPDLGISMNDTTTGSEEDERDNNSDDPREGAIDPSNRRPRGRP
PGSKNKPKPPIFVTRDSPNALRSHVMEVASGTDIAESIAQFSRKRQRGVCVMSASGTVMNVTLRQP
SAPGSVMALQGRFEILSLTGAFLPGPSPPGSTGLTIYLAGGQGVVGGSVVGSVLVAGPVMVIAAT
FSNATYERLPVEEEEEADTVTPGLGGGSPFQLGMGDQNPAGYNMQPNLIPNGGQMNHFAFALA
HGRPTY

FIGURE 6 (continued)

SEQ ID NO: 17 Lotus japonicus AHL19/20 nucleic acid sequence AP004971

ATGGCTAATCCTTGGTGGACAAGCCAGGGAGGGTTCTCTGGGGTTGACCCAGGAACCCATTACCT
 GGCTTGAGCAAACGTCACACGGACCTTGTGATCAATGAAAACAGCAGCGGTGGTAATAGAGATGAA
 GATGAAGATGATAACAGGGAAGATGAGCCAAAAGAAGGTGCAGTTGAGGTTGGAACTCGGAGACCA
 AGGGGAAGACCACCGGGATCCAAGAACAAGCCAAGACCACCCATCTTTGTAACAAGGGACAGCCCA
 AACGCCCTGAGGAGTCATGTTATGGAGGTTGCAGGAGGAGCTGATGTCGCAGAAAGCGTGGCCAG
 TTTGCGAGGAGGCGCCAGCGTGGGGTTTGTGTGATGAGCGGGAGTGGCTCTGTGGCAAACGTTACC
 CTGAGACAACCTGCGGCTCCGGGTGCTGTTGTAGCACTCCATGGCAGGTTTGAGATCTTATCCCTA
 ACTGGGGCGTTCCCTACCTGGCCCTGCTCCTCCAGGATCCACTGGTCTAACAGTGTATCTTTCTGGA
 GGACAGGGTCAGGTAGTGGGAGGGAGTGTGGTGGGGTCTCTAGTTGCAGCAGGACCAGTTATGGTC
 ATTGCTGCAACTTTTGTCTAATGCAACATATGAGAGGTTGCCACTTGATGATGATGATGAGGGACCT
 AGTGGGGCCGCTACGGCGGCAAGCGGAGGAGGAAGTGGATCGTCTCCTCCACCTGGAATTGGAATT
 GGCAGTGGTGGGGGTCACTCAACTGCAGGCTGGACTGGTTCCAGATCCATCATCCATGCCGTTGTAT
 AATCTGCCACCAAATCTGTTGTCCAATGGAGGAGGAGGACAAGTGGGGCATGATGCTCTTGCTTGG
 GCTCATGGAAGAACACCTTACTGA

SEQ ID NO: 18 Lotus japonicus AHL19/20 translated polypeptide sequence

MANPWWTSQGGFSGVDPGTHSPGLSKRHTDLVINENSSGGNRDEDEDDNREDEPKEGAVEVGTRRP
 RGRPPGSKNKPRPPIFVTRDSPNALRSHVMEVAGGADVAESVAQFARRRQRGVCVMSGSGSVANVT
 LRQPAAPGAVVALHGRFEILSLTGAFPLPGPAPPSTGLTVYLSGGQGQVVGGSVVGSLVAAGPVMV
 IAATFANATYERLPLDDDDGPGSAATAASGGSGSSPPPGIGIGSGGGHQLQAGLVPDPSSMPPLY
 NLPPNLLSNGGGGQVGHDAALAWAHGRTPY

SEQ ID NO: 19 Oryza sativa AHL19/20 nucleic acid sequence AK110263 Os08g0563200

ATGGCGTCCAAGGAGCCAAGCGGCGACACGACACGAGATGAACGGGACCAGCGCCGGGGGCGGC
 GAGCCCAAGGACGGCGCGGTGGTGACCGGCCGCAACCGGCGCCCCCGCGGACGGCCGCCGGGCTCC
 AAGAACAAGCCCAAGCCGCCCATCTTCGTGACGCGGGACAGCCCGAACGCGCTGCGCAGCCACGTC
 ATGGAGGTGGCCGGCGGCGCGATGTGCGCGAGTCCATCGCGCACTTCGCGCGGCGGCGGCAGCGC
 GGCGTCTGCGTGCTCAGCGGGGCGGCACCGTGACCGACGTGGCCCTGCGCCAGCCGGCCGCGCCG
 AGCGCCGTGGTGGCGCTCCGTGGGCGGTTTCGAGATCCTGTCCCTGACGGGGACGTTCCCTGCCGGGG
 CCGGCGCCGCCGGGCTCCACCGGGCTGACCGTGTACCTCGCCGGCGGGCAGGGGACAGGTGGTGGGC
 GGCAGCGTGGTGGGGACGCTCACCGCGGCGGGGCGGTCATGGTGATCGCCTCCACCTTCGCCAAC
 GCCACCTACGAGAGGCTGCCGCTGGATCAGGAGGAGGAGGAAGCAGCGGCAGGCGGCATGATGGCG
 CCGCCGCCACTCATGGCCGGCGCCGCCGATCCACTACTTTTCGGCGGGGAATGCACGACGCCGGG
 CTGCTGCATGGCACCATGCCCGCCCTCCGCCGCCGCCGCCCTACTAG

SEQ ID NO: 20 Oryza sativa AHL19/20 translated polypeptide sequence

MASKEPSGDHDEMNGTSAGGGEPKDGAVVTGRNRRPRGRPPGSKNPKPPIFVTRDSPNALRSHV
 MEVAGGADVAESIAHFARRRQRGVCVLSGAGTVTDVALRQPAAPSAVVALRGRFEILSLTGTFPLPG
 PAPPSTGLTVYLAGGQGQVVGGSVVGTLTAAGPVMVIASTFANATYERLPLDQEEEEAAAGGMMMA
 PPPLMAGAADPLLFGGGMHDAGLAAWHHARPPPPPPY

FIGURE 6 (continued)

SEQ ID NO: 21 *Oryza sativa* AHL19/20 II nucleic acid sequence CT837915 Os02g0820800

ATGGGCTTGCCGGAGCAGCCGTCCGGCTCGTCGGGCCCCAAGGCGGAGCTCCCGGTGGCCAAGGAG
CCGGAGGCGAGCCCCGACGGGGGGCGCGGCGGCGGACACGCCGACGAGAACAACGAATCCGGCGGC
GGCGAGCCGCGGGAGGGCGCCGTGGTGGCGGCGCCCAACCGGCGCCCCCGCGGCCGCCCGCGGGC
TCCAAGAACAAGCCGAAGCCGCCATCTTCGTGACGCGGACAGCCCCAACGCGCTGCGCAGTCAC
GTCATGGAGGTGGCCGGCGGCGCCGACGTCGCCGACGCCATCGCGCAGTTCTCGCGCCGCCGCCAG
CGCGGCGTCTGCGTGCTCAGCGGCGCCGGGACGGTCGCCAACGTCGCGCTGCGCCAGCCGTCGGCG
CCCCGGCGCCGTGTCGCCCTGCACGGCCGCTTCGAGATCCTCTCCCTCACCGGCACCTTCCTCCCA
GGCCCCGGCGCCTCCGGGTTCACGGGGCTCACCGTCTACCTCGCCGGCGGCCAGGGCCAGGTTGTC
GGCGGCAGCGTCTGTTGGGTGCTCATCGCCGCGGGCCCGGTCATGGTGATCGCGTCCACGTTCCGC
AACGCCACCTACGAGCGCCTGCCACTGGAGGAAGAAGAGGAGGGCTCAGGCCCCGCCATGCCCGGC
GGCGCCGAGCCCCCTCATGGCCGGCGGCCACGGCATCGCCGACCCTTCGGCGCTGCCAATGTTCAAC
CTGCCGCCGAGCAACGGGCTCGGCGGCGGCGGCGACGGCTTCCCATGGGCGGCGCACCCCTGCCCA
CCGTACTGA

SEQ ID NO: 22 *Oryza sativa* AHL19/20 II translated polypeptide sequence

MGLPEQPSGSSGPKAELPVAKEPEASPTGGAAADHADENNESGGGEPREGAVVAAPNRRPRGRPPG
SKNKPKPPIFVTRDSPNALRSHVMEVAGGADVADIAIQFSRRRQRGVCVLSGAGTVANVALRQPSA
PGAVVALHGRFEILSLTGTFLPGPAPPGSTGLTVYLAGGQGQVVGGSVVGSLIAAGPVMVIASTFA
NATYERLPLEEEEEEGSGPPMPGGAELPMAGGHGIADPSALPMFNLPPSNGLGGGGDGFPAAHPCP
PY

SEQ ID NO: 23 *Populus tremuloides* AHL19/20 nucleic acid sequence scaff_XIII.441

ATGGCĀAACCGGTGGTGGACAGGGCAAGTGGGATTGCCGGGGATGGACACATCAACCAGTTCATCA
TCTCCAATGAAAAAGCCAGATCTAGGTATATCCATGTCCAACAACAATAGAGAAGCCACCGAGAGT
GGTGCTGGCAAAGAAGATGAGCAAGAAGACGAAAGAGAAAAATAGCGACGAGCCTAGAGAAGGCGCT
ATAGATATCGCCTCTCGCCGCCCTAGAGGCCGTCCACCAGGGTCCAAGAACAAGCCTAAGCCACCA
ATTTTCGTTACTCGAGACAGCCCTAATGCACTCAAGAGTCATGTGATGGAGATAGCTAGTGGATCT
GATATAGCTGAAAATTTAGCTTGTTTTGCAGGAAGAGACAAAGAGGAGTTTGTGTGCTTAGTGGA
AGTGGTATGGTAACCAATGTAACCCTCAAGCAACCTTCTGCCTCAGGTGCTGTTATGGCTCTCCAT
GGTAGGTTTGAGATTTTGTCACTCACTGGAGCGTTCTTGCTGACCAGCCCCACCTGGAGCGACA
GGACTAACTATATATTTAGCCGGAGGGCAAGGACAAGTGGTAGGAGGCAGTGTGGTAGGATCACTA
GTTGCATCAGGACCGGTAATGGTTATTGCTGCAACATTTTCAAATGCTACTTATGAGAGATTGCCA
CTAGAAGATGAAGAGGAAGGCAGTGGTGGCGCACAAGGGCAGCTCGGTGGCGGCAACGGTAGCGGT
GAGGGTAATGGTGGGGGCATGGGGGATCCAGCAACATCAATGCCAGTTTATCAATTGCCAAATATG
GTGCCTAATGGACAATTGAACCATGAAGGATATGGGTGGGCTCACGGCAGACCACCCTATTAG

SEQ ID NO: 24 *Populus tremuloides* AHL19/20 translated polypeptide sequence

MANRWWTGQVGLPGMDTSTSSSPMKKPD LGI SMSNNNREATESGAGKEDEQEDERENSDEPREGA
IDIASRRPRGRPPGSKNKPKPPIFVTRDSPNALKSHVMEIASGSDIAENLACFARKRQRGVCVLSG
SGMVTNVTLKQPSASGAVMALHGRFEILSLTGAFLPGPAPPGATGLTIYLAGGQGQVVGGSVVGSL
VASGPVMVIAATFSNATYERLPLEDEEEEGSGGAQGQLGGGNGSGEGNGGGMGDPATSMFVYQLPNM
VPNGQLNHEGYGWAHGRPPY

FIGURE 6 (continued)

SEQ ID NO: 25 *Solanum tuberosum* AHL19/20 nucleic acid sequence, contig of CN215397.1, CK276075.1

ATGTCAAACCCATGGTGGACAGGCCAAGTAGGTTTACAAGGAGTTGAAACATCATCATCCGCGGGT
 TCGCCTTCTCTCAAGAAGCCAGATCTAGGCGTATCAATGAACGATATAGTGGGTGGTAGTGGTAGT
 CATGATGAAGATAGGGACCATAGCGACGACCCTAAAGAGGGTGCAGTCGAAGTAGCCACTCGTCGA
 CCCAGAGGTCGACCAGCTGGCTCAAAGAACAACCTAAACCACCAATATTTGTTACAAGGGATAGC
 CCTAACGCACTTAGAAGCCACGTAATGGAAGTTGCTAATGGAGCTGATGTGGCGGAAAGTATAGCT
 CAATTTGCTAGGAAAAGACAAAGAGGTGTTTGTGTTTTGAGTGCTACTGGAAGTGTACTAATGTA
 ACCCTAAGACAACCATCTGCTCCTGGAGCTGTCATGGCATTACACGGCCGGTTCGAGATCTTATCG
 TTGACCGGAGCTTTCTTACCTGGACCCGCCCTCCTGGATCAACAGGGTTGACTATATACCTAGCA
 GGAGGACAAGGACAAGTTGTGGGAGGAAGTGTAGTAGGGTCTTTAGTGGCTTCCGGACCAGTTATG
 GTAATTGCATCAACTTTTTTTAATGCAACATATGAGAGGCTACCTTTGGAGGAGGAGGAAGAAGGC
 GGTGGAACGGTGGCCCAAGGACAACCTGGTGGTGGTGGATCGCCACCGGGAATGGGAGGAAGTGGT
 GGTGGTGGTGGAGGACAACAACAAGGTGGTGGTGGTATGGGTGATATTCATCATCAAATATG
 CCAGTATATAATTTGCCACCAAATTTGCTACCAAATGGTGGACAAATGAACCATGAAGCATTGGT
 TGGGCACATGGACGCCCTCCTTTTTAA

SEQ ID NO: 26 *Solanum tuberosum* AHL19/20 translated polypeptide sequence

MSNPWWTGQVGLQGVETSSSAGSPSLKKPDLGVSMNDIVGGSGSHDEDRDHSDDPKEGAVEVATRR
 PRGRPAGSKNPKPPIFVTRDSPNALRSHVMEVANGADVAESIAQFARKRQRGVCLSATGTVTNV
 TLRQPSAPGAVMALHGRFEILSLTGAFPLPGPAPPGSTGLTIYLAGGQGVVGGSVVGSVLVASGPVM
 VIASTFFNATYERLPLEEEEEGGGTVAQGQLGGGGSPPGMGGSGGGGGGQQQQGGGGMGDIPISSNM
 PVYNLPPNLLPNGGQMNHEAFGWAHGRPPF

SEQ ID NO: 27 *Thlaspi caerulescens* AHL19/20 nucleic acid sequence DQ022564

ATGGCGAATCCATGGTGGACAGGACAAGTGAATCTCTCCGGCCTTGAAACGACGCCCGCTGGTTCC
 TCTCAGTTAAAGAAATCAGATCTCCACATCTCCATGAACATGGCCATGGACTCAGGTCATAACAAC
 CATCATCATCACCAAGAAGTCGACAACAATAACAACAACGATGACGACAGAGATAACTTGAGCGGC
 GATGAACACGAGCCACGTGAAGGAGCCGTAGAAGCCCCACGCGCCGTCCACGTGGACGTCCTGCT
 GGTTCCAAAGAACAACCAAAGCCACCGATCTTTGTACGCGCGATTCTCCAAACGCTCTCAAGAGC
 CATGTGATGGAGATCGCTAGTGGGACTGACGTCATCGAAACCCTAGCTACTTTGCTAGGCGGCGC
 CAACGTGGCATCTGCATCTTGAGCGGCAACGGCACGGTGGCTAACGTCACTCTCCGCCAACCATCA
 TCTGCCGCAGTTGCTGCGGCTCCCGGGGGTGC GGCGGTTTGGCTTTACAAGGGAGGTTTGAGATT
 CTCTCTTTAACAGGATCGTTCTTGCCCTGGACCTGCTCCACCTGGATCCACCGGTTTAACCATCTAC
 TTAGCCGGTGGTCAAGGTCAGGTCGTTGGAGGAAGTGTGGTGGGGCCATTGATGGCGGCTGGTCCG
 GTTATGTTAATCGCGGCCACGTTTTCTAATGCGACTTACGAGAGATTGCCTTTGGAGGAGGAAGAG
 GCGGCTGAGAGAGGCGGTGGAGGAGGCAGCGTCCCAGGACAACCTCGGAGGGGGAGGCTCGCCGCTG
 AGTAGCGGTGGTGGTGGAGGGGATGGCAATCAAGGACTTCCGGTGTACAATATGCCCGGAAATCTT
 GTTTCTAATGGTGGCGGAGGCGGAGGACAGATGAGTGGCCAAGAAGCTTATGGTTGGGCTCAAGCT
 AGGTCAGGATTTTAA

SEQ ID NO: 28 *Thlaspi caerulescens* AHL19/20 translated polypeptide sequence

MANPWWTGQVNLSGLETPPGSSQLKKSDLHISMNMAMDSGHNNHHHHQEVDNNNNNDDDRDNLSG
 DEHEPREGAVEAPTRPRGRPAGSKNPKPPIFVTRDSPNALKSHVMEIASGTDVIETLATFARRR
 QRGICILSGNGTVANVTLRQPSSAAVAAAPGGAVALALQGRFEILSLTGSFLPGPAPPGSTGLTIY
 LAGGQGVVGGSVVGPLMAAGPVMLIAATFSNATYERLPLEEEEEAAERGGGGGSPVQQLGGGGSPL
 SSGGGGGDGNQGLPVYNMGPGLVSNGGGGGGQMSGQEAYGWAQARSGF

FIGURE 6 (continued)

SEQ ID NO: 29 Vitis vinifera AHL19/20 nucleic acid sequence AM463589

ATGGCGAACCGGTGGTGGGCTGGGCAGGTGGGTCTGCAAGGTGTAGATACCTCATCAGCTTCACCT
 GCAATGAAGAAACCAGATCTGGGAATATCCATGAATGAAAATGGAGGAAGCGGGAGCGGAGGCGGA
 GGAGAGGAAGAAGAGGAAAAAGAAAACAGTGATGAGCCCAGAGAGGGTGCAATTGAGGTGGCTACG
 CGCAGGCCTAGGGGCGCGCCCTGGCTCCAAGAACAAGCCAAAACCTCCGATTTTTGTGACAAGG
 GACAGCCCTAACGCTCTGCGCAGCCACGTTATGGAGGTGGCAAACGGCTCCGACATCACAGAAAGC
 ATAGCCCAATTTCGCGAGAAGGCGGCAACGAGGCGTCTGCGTGCTCAGCGCAAGTGGGACAGTCATG
 AACGTAACGCTTCGCCAGCCTTCTGCCCCCTGGTGGTGCAGTTATGGCACTTCATGGCCGATTTCGAA
 ATTCTTTCTTAACCGGCGCGTTCCTACCGGGACCAGCGCCACCAGGCTCCACTGGACTAACCATA
 TACCTAGCAGGCGGTCAAGCTCAGGTCGTGGGTGGTAGCGTGGTGGGTTCACTCATAGCGGCAGGT
 CCAGTTATGGTGATTGCAGCTACCTTTTCGAATGCAACCTACGAGAGGCTCCCCCTAGAAGACGAA
 GAAGAGGCGGGCAGCGCAGCACAGGAGCAGCTCGCTGGCGGCGGAGGCGGTGGTGGGTCAACGCCA
 GGGATTGGCGGCAGTGGGGGGCAGCAGCAGGAGGATGGCAGATCCTTCTCCATGCCGGTTTAT
 AATTTGCCACCAAATTTGCTTCCAAATGGTGGACAACCTGAACCATGATGCTTATGGTTGGGCACAT
 GGGCGCCAGCCTTACTAG

SEQ ID NO: 30 Vitis vinifera AHL19/20 translated polypeptide sequence

MANRWAGQVGLQGVDTSASPAMKKPDLGISMNENGSGSGGGGEEEEEEKENSDEPREGAIEVAT
 RRPRGRPPGSKNPKPPIFVTRDSPNALRSHVMEVANGSDITESIAQFARRRQRGVCVLSASGTVM
 NVTLRQPSAPGGAVMALHGRFEILSLTGAFLPGPAPPGSTGLTIYLAGGQAQVVGSSVVGSLIAAG
 PVMVIAATFSNATYERLPLEDEEEAGSAAQEQLAGGGGGGSGSPGIGSGGQQQAGMADPSSMPVY
 NLPPNLLPNGGQLNHDAYGWAHGRQPY

SEQ ID NO: 31 Vitis vinifera AHL19/20 II nucleic acid sequence AM429692

ATGGACCCGGCAGCTGTTTCGCCGATGCTAAATAAACGCGATCGCGAGATATCAATCAACGATAAC
 CCCGGCACAGGAGACGATGAAGAAGAGAAAAGACAACGAAGGCGAGCCACCGGAGGGTGCAGTAGAA
 GTCGGCACTCGTAGACCAAGAGGTCGCCCCCTGGATCCAAAAACAAGCCCAAACCCCCCTATTTTC
 GTCACGCGCGACAGCCCGAACGCCCTTCGGAGCCACGTGATGGAGGTGGCCGGCGGCCACGACGTT
 GCCGAAAGCGTCGCCCAGTTTCGCCCGTAGGCGTCAACGAGGGGTCTGCGTCCCTCAGCGGCAGCGGC
 TCCGTAGCCAACGTGACTCTGAGACAGCCCGCGCGCTGGCGCCGTGGTGGCACTCCATGGAAGA
 TTCGAGATTCTGTCCCTAACAGGAGCATTCCTCCCCGGACCTGCCCCCTCCCGGCTCCACTGGACTC
 ACCGTGTACCTCGCCGGAGGTCAGGGCCAGGTTGTGGGAGGAAGTGTGGTTGGATCACTGGTAGCG
 GCAGGCCCCGGTGATAGTGATAGCCGCCACTTTTGCGAACGCAACATACGAAAGACTGCCTCTGGAA
 GAAGAAGAAGAAGGTGGGCAGGCGCCGCCGCGAGTGGTTCGCCGCGCTGCAATTGGAAGCAGTGGT
 GGACAGCATCACTCTGGCCTGCCGGAGCTGCCCATATACAATCTGCCACCGAACCTACTCCCTAAC
 GCGGCCCAATTGAGTCATGACCCCTACTCATGGGCTCATGCTCGGCCCCCTTACTGA

SEQ ID NO: 32 Vitis vinifera AHL19/20 II translated polypeptide sequence

MDPAAVSPMLNKRDRDISINDNPGTGDDEEEKDNEGEPTGEGAVEVGTRRPRGRPPGSKNPKPPIF
 VTRDSPNALRSHVMEVAGGHDVAESVAQFARRRQRGVCVLSGSGSVANVTLRQPAAPGAVVALHGR
 FEILSLTGAFLPGPAPPGSTGLTVYLAGGQGVVVGSSVVGSLVAAGPVIVIAATFANATYERLPLE
 EEEEGGQAPPPSGSPPAIGSSGGQHHSGLPELPIYNLPPNLLPNGGQLSHDPYSWAHARPPY

FIGURE 6 (continued)

SEQ ID NO: 33 Zea mays AHL19/20 nucleic acid sequence AC190270

ATGGCACCTTCCTCCAAGGACGGCGCCACCGCCACCGAGCAGCCGACGAGCGGGCAGCAGCAGCGG
 GAGAACGGCGGCACGGGCGAGCCCAAGGAAGGCGCGGTGGTGGCGGGCAACCGGCGGCCCCGCGGG
 CGGCCGCGGGGTCCAAGAACAAGCCCAAGCCGCCATCTTCGTGACGCGCAGCAGCCCAACGCG
 CTGCGCAGCCACGTGATGGAGGTGGCCGGCGGCGCCGACGTGGCCGAGTCCATCGCCCACTTCGCG
 CGCCGCAGGCAGCGCGGCGTGTGCGTGCTCAGCGGCGCGGGCACCCTCGCCGACGTGGCGCTCCGC
 CAGCCCGCGGCTCCGGGCGCCGTGGTCCGCTCCGCGGCGCTTCGAGATCCTCTCGCTCACCGGC
 ACGTTCCTGCCGGGCCCCGCGCCGGGCTCCACGGGGCTCACCCTGTACCTCGCGGGCGGCCAG
 GGGCAGGTTCGTCGGCGGCAGCGTCGTCGGCACGCTCACCAGCGCGGGGCCCCGTCATGGTGATGGCG
 TCCACGTTTCGCAACGCCACCTACGAGAGGCTGCCGCTGGACGACGCCGACGAGGAGCCCCCGGG
 CAGCAGGCGGGCAGCTGCCTCCCGACCGGGCGGAGGGCAGCCTATGGTAATGGGCGGGATGGCC
 GACCCCTCAGCGGTGCCAATGTTTCGGCGGCGCCGGCGGTGTGCCGCCAAGCCTCATGCCAGCAGGG
 GCCGCAGCCGCTCCTCCGGTGCGGGCCTGCAGCTCGGGCACGACCGACTTGTCATGGGCTCATGCA
 CGGCCACCGCCATACTAG

SEQ ID NO: 34 Zea mays AHL19/20 translated polypeptide sequence

MAPSSKDGAATEQPTSGDDRENGGTGEPKEGAVVAGNRPRGRPPGSKNPKPPIFVTRDSPNA
 LRSHVMEVAGGADVAESIAHFARRRQRGVCVLSGAGTVADVALRQPAAPGAVVALRGRFEILSLTG
 TFLPGPAPPGSTGLTVYLAGGQGVVGGSVVGLTLTAAGPVMVMASTFANATYERLPLDDADEEPAG
 QQAQLPPGPGGGQPMVMGMDPSAVPMFAGGVPVPSLMPAGAAAASSGAGLQLGHDRLOWAHA
 RPPPY

SEQ ID NO: 35 Oryza sativa GOS2 promoter

AATCCGAAAAGTTTCTGCACCGTTTTACCCCCCTAACTAACAATATAGGGAACGTGTGCTAAATAT
 AAAATGAGACCTTATATATGTAGCGCTGATACTAGAACTATGCAAGAAAACCTCATCCACCTACT
 TTAGTGGCAATCGGGCTAAATAAAAAAGAGTCGCTACACTAGTTTCGTTTTCTTAGTAATTAAGT
 GGGAAAATGAAATCATTATTGCTTAGAATATACGTTACATCTCTGTGCATGAAGTTAAATTATTTCG
 AGGTAGCCATAATTGTCATCAAACTCTTCTTGAATAAAAAATCTTTCTAGCTGAACCTCAATGGGT
 AAAGAGAGAGATTTTTTTTAAAAAATAGAATGAAGATATTCTGAACGTATTGGCAAAGATTTAAA
 CATATAATTATATAATTTTATAGTTTGTGCATTTCGTATATCGCACATCATTAAAGGACATGTCTTA
 CTCCATCCCAATTTTTATTTAGTAATTAAGACAATTGACTTATTTTTATTATTTATCTTTTTTCG
 ATTAGATGCAAGGTACTTACGCACACACTTTGTGCTCATGTGCATGTGTGAGTGCACCTCCTCAAT
 ACACGTTCAACTAGCAACACATCTCTAATATCACTCGCCTATTTAATACATTTAGGTAGCAATATC
 TGAATTCAAGCACTCCACCATCACCAGACCACTTTAATAATATCTAAAATACAAAAAATAATTTT
 ACAGAAATAGCATGAAAAGTATGAAACGAACATTTAGGTTTTTCACATACAAAAAAGAAATT
 TTGCTCGTGCAGCGAGCGCAATCTCCCATATTGGGCACACAGGCAACAACAGAGTGGCTGCCCCA
 GAACAACCCACAAAAACGATGATCTAACGGAGGACAGCAAGTCCGCAACAACCTTTTAACAGCAG
 GCTTTGCGGCCAGGAGAGAGGAGAGGCAAGAAAACCAAGCATCCTCCTTCTCCCATCTATAA
 ATTCTCCCCCTTTTCCCCTCTCTATATAGGAGGCATCCAAGCCAAGAAGAGGGAGAGCACCAG
 GACACGCGACTAGCAGAAGCCGAGCGACCGCCTTCTCGATCCATATCTTCCGGTCGAGTTCTTGGT
 CGATCTCTTCCCTCCTCCACCTCCTCCTCACAGGTATGTGCCTCCCTTCGGTTGTTCTTGGATT
 ATTGTTCTAGGTTGTGTAGTACGGGCGTTGATGTTAGGAAAGGGATCTGTATCTGTGATGATTCC
 TGTTCTTGGATTGTTGGGATAGAGGGGTTCTTGATGTTGCATGTTATCGGTTCCGTTGATTAGTAGT
 ATGGTTTTCAATCGTCTGGAGAGCTCTATGGAATGAAATGGTTTAGGGATCGGAATCTTGCGATT
 TTGTGAGTACCTTTTGTGTTGAGGTAAAATCAGAGCACCGGTGATTTTGCTTGGTGTAATAAGTAC
 GGTGTTGTTGGTCTCGATTCTGGTAGTGATGCTTCTCGATTGACGAAGCTATCCTTTGTTTATTC
 CCTATTGAACAAAAATAATCCAACCTTGAAGACGGTCCCGTTGATGAGATTGAATGATTGATTCTT
 AAGCCTGTCCAAAATTCGCAGCTGGCTTGTTTAGATACAGTAGTCCCCATCACGAAATTCATGGA

FIGURE 6 (continued)

AACAGTTATAATCCTCAGGAACAGGGGATTCCCTGTTCTTCCGATTTGCTTTAGTCCCAGAATTTT
TTTTCCCAAATATCTTAAAAAGTCACTTTCTGGTTCAGTTCAATGAATTGATTGCTACAAATAATG
CTTTTATAGCGTTATCCTAGCTGTAGTTTCACTTAATAGGTAATACCCCTATAGTTTATGTCAGGAGA
AGAACTTATCCGATTTCTGATCTCCATTTTAAATTATATGAAATGAACTGTAGCATAAGCAGTATT
CATTTGGATTATTTTTTTTATTAGCTCTCACCCCTTCATTATTCTGAGCTGAAAGTCTGGCATGAA
CTGTCCTCAATTTTGTGTTTCAAATTCACATCGATTATCTATGCATTATCCTCTTGTATCTACCTGT
AGAAGTTTCTTTTTGGTTATTCCTTGACTGCTTGATTACAGAAAGAAATTTATGAAGCTGTAATCG
GGATAGTTATACTGCTTGTCTTATGATTTCATTTCTTTGTGCAGTTCTTGGTGTAGCTTGCCACT
TTCACCAGCAAAGTTC

SEQ ID NO: 36 Conserved Domain comprised in SEQ ID NO: 2

EPREGAVEAPTRRPRGRPAGSKNPKPPIFVTRDSPNALKSHVMEIASGTDVIETLATFARRRQRG
ICILSGNGTVANVTLRQPSTA AVAAAPGGA AVLALQGRFEILSLTGSFLPGPAPPGSTGLTIYLAG
GQGQVVGGSVVGPLMAAGPVMLIAATFSNATYERLPLEEEE

SEQ ID NO: 37 AT hook

RRPRGRP (P/A) GS (K/R) NKP

SEQ ID NO: 38 PPC domain (DUF296) comprised in SEQ ID NO: 2

LKSHVMEIASGTDVIETLATFARRRQRGICILSGNGTVANVTLRQPSTA AVAAAPGGA AVLALQGR
FEILSLTGSFLPGPAPPGSTGLTIYLAGGQGQVVGGSVVGPLMAAGPVMLIAATFSNAT

SEQ ID NO: 39 prm8135

GGGGACAAGTTTGTACAAAAAAGCAGGCTTAAACAATGGCGAATCCATGGTG

SEQ ID NO: 40 prm8136

GGGGACCACTTTGTACAAGAAAGCTGGGTAAAAACCATTTTAACGCACG

SEQ ID NO: 41 Brassica oleracea AHL19/20 nucleic acid sequence

Atgcgaaatccatggtggacaggacaagtgaatctctccagtctcgaaacgacgcgcgcgagttcc
tctcagttaaagacaccagatctccacatctccatgaacatggccatggtctcaggtcataaacaac
caccatcatcatcaccaagaagtcaacaccaacaacaacgaagacgatagagacaacttgagc
ggcgacgaccgcgagccacgtgaaggagccgtggaagctcccacgcgcgcgaccacgtggacgtcct
gctggttccaagaacaaaccaaagccaccaatctttgtcacgcgtgattctccaaacgctctcaag
agccatgtcatggagatcgctagtgggactgatgtcatagaaaccctagctactttcgctaggcgg
cgccaacgtggcatctgcatcttgagcggtaacggcacggtgggtaacgtcacactccgtcaacca
tcagtggctcccgttgacgtgcccctgggtgggtgcggtgtattggcggtacaagggaggtttgag
attctttctctaaccggttctttcttacctggaccggtccacctggatccactggtttaactatt
tacttagctggtggtcaaggtcaggttgttgagggaagcgtggtggggcattgatggctgctggt
ccggtgatgctaatacgctgccacgttttctaatacgacttatgagagattacctttggatgaggaa
gaagcggctgaaagaggtggcgggtggaagcgacggaggagtgggtccagggcagctcgggggcgta
ggttccccgctgagtagtggtggcgggtggaggccacgggaaccaaggacttcccgcatataatatg
cccgaaaaccttgcttctaataggcgggtggaggaggacagatgagcagccaagaagcgtacggttgg
gctcaagctaggtcaggatttta

SEQ ID NO: 42 Brassica oleracea AHL19/20 translated polypeptide sequence

Mrnpwwtgqvnllslettpssssqlktpdlhismnmamvsgnnhhhhhqevntnnnnneddrdnl
gddrepegaveaptrrprgrpagsknkpkppi fvtrdspnalkshvmeiasgtdvietlatfarr
rrgicilsgngtvavnlrqp svapvaaapgga avlallqgrfeilsltgsflpgpappgstgli

ylaggggqvvggsvvgalmaagpvmliaatfsnatyerlpldeeeaaerggggsdggvvpqqlggv
 gsplssggggghgnqglpaynmpgnlasnggggqmssqeygwaqarsgf

SEQ ID NO: 43 *Medicago truncatula* AHL19/20 nucleic acid sequence

Atggcgaaacaggtggtggaccggaccggttgggtctaggaggatggacaactcagtaacctcctct
 ccactaggaaaaccggatctgggtttctccatgaatcaaagtgctgtaacaggagtgaacaacatg
 aacaacaacaacaatgaagaagaagaagatgagaaagaaaacagcgacgaacacaaaggaggtgca
 atagaaacaaacacctccacgcgccgcccgaaggccgtccatcagggttcaaaaaacaaacaaaa
 ccaccaatattcataacaagagatagccctaacgcgctacgaagccatgtcatggaagtagcaaca
 ggaacagatatatcagatagcatcggttcagtttgcaagaaaaagacagagaggtatttgcatctta
 agcgcaagtggaaaccgctcgtaacgtttctctccggcaacctacagggtcccggagctgtggttagcg
 cttccaggggagatttgatatactctctttgactggttctgtgcttcctggaccttcaccgccggga
 gctactggtttgactatttatctttctggaggacaaggacaggtggttggcgccggagttgttggt
 ccccttggtggcgccaggaccagttatggtgatggcgccgacattttcgaatgctacgtatgagagg
 ctgccggttgaggatggtgatgatcaagaaggccatcagggtggtggtggtgatgatgagtctccg
 acgcgtgcagcggggatgggacagttagcgattggatctgttggagaaggttcttcaattccacca
 ggctataacaatggttggtggttaatttgggtgtttcaaattggaggacaacaacaattggtgaataat
 catgaggcttataataattctccttgggggtcatgctagtcatggttagaccaccataactaa

SEQ ID NO: 44 *Medicago truncatula* AHL19/20 translated polypeptide sequence

manrwwtgpvglggmdnsvtssplgkpdlgfsmnqsavtgvnnmnnnnneeeedekensdehkgga
 ietntstrrprgrpsgsknkpkipifitrdspnalrshvmevatgtddisdsivqfarkrqrqgicil
 sasgtvvnvslrqptgpgavvalpgrfdilsltgsvlpgpsppgatgltiylsggggqvvggsvvg
 plvaagpvmliaatfsnatyerlpvedgddqeghqgggddespttraagmgqlaigsvgegssipp
 gynnvgnlgvsnnggqqqlnnheaynnspwghashgrppy

FIGURE 6 (continued)

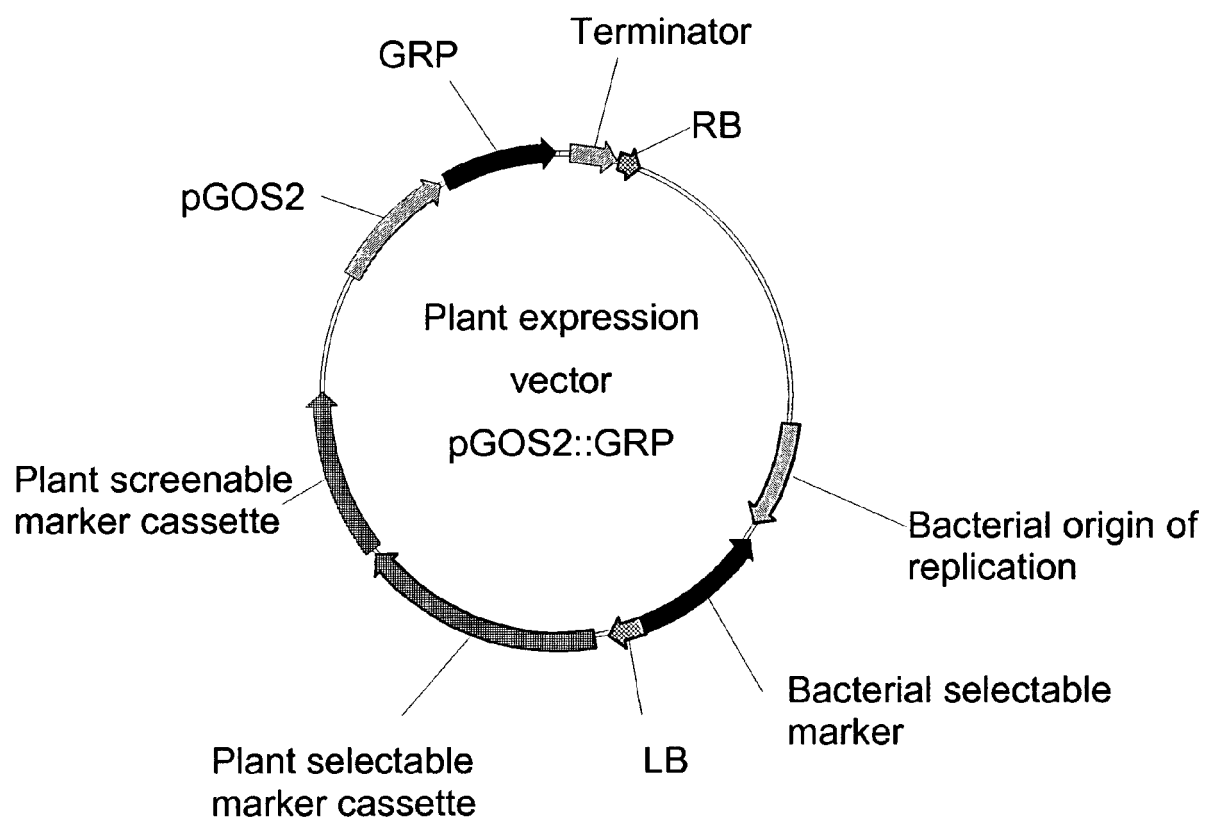


FIGURE 7

SEQ ID NO: 45, *Arabidopsis thaliana* MT2A nucleic acid sequence

gcagttccctactctcgcgttaacgctagcatggatctcgggccccaaataatgatttttattttga
 ctgatatgtacacctgttcggttgcaacaaattgatgagcaatgctttttataatgccaaactttgtac
 aaaaaagcaggcttcacaatgtcttgctgtggaggaaactgcggatgtggatctggctgcaagtgc
 ggcaacggttggtggaggttgcaaaatgtacctgacttgggattctccggcgagacaaccacaact
 gagacttttgtcttggcggttgacccggcgatgaagaatcagtacgaggcttcaggggagagtaac
 aacgctgagaacgatgcttgcaagtgtggatctgactgcaagtgtgatccttgcaacctgcaagtga
 aaccagctttcttgtacaaagttggcattataagaaagcattgcttatcaatttggtgcaacgaa
 caggctactatcagtcaaaaataaaatcattatttgccatccagctgcagctctggcccggtgtctca
 aaatctctgatgttacattgcacaagataaaaaatatatcatcatgaacaataaaaactgtctgctta
 cataaacagtaataacaaggggtgttatgagccatattc

SEQ ID NO: 46, *Arabidopsis thaliana* MT2A polypeptide sequence

MSCCGGNCGCGSGCKGNGCGGCKMYPDLGFSGETTTTETFVLGVAPAMKNQYEASGESNNAENDA
 CKCGSDCKCDPCTCK

SEQ ID NO: 47, *Oryza sativa* GOS2 promoter

aatccgaaaagtttctgcaccggttttcaccccctaactaacaatatagggaaacgtgtgctaaatat
 aaaatgagaccttatatatgtagcgctgataactagaactatgcaagaaaaactcatccacctact
 ttagtggaatcgggctaaataaaaaagagtcgctacactagtttctgttttcttagtaatttaagt
 gggaaaatgaaatcattattgcttagaatatacgttcacatctctgtcatgaagttaaattattcg
 aggtagccataattgtcatcaaaactcttcttgaataaaaaaatcttcttagctgaactcaatgggt
 aaagagagagatttttttttaaaaaatagaatgaagatattctgaacgtattggcacaagatttaaa
 catataattatataatttttatagtttgtgcattcgtcatatcgcacatcattaaggacatgtctta
 ctccatcccaattttttattagtaattaaagacaattgacttatttttattattttatcttttttcg
 attagatgcaaggtaacttacgcacacactttgtgctcatgtgcatgtgtgagtgcaacctcctcaat
 acacgttcaactagcaacacatctctaataatcactcgcctattttaatacatttaggtagcaatatac
 tgaattcaagcactccaccatcaccagaccacttttaataatatctaaaaatacaaaaaataatttt
 acagaatagcatgaaaagtatgaaacgaactatttaggtttttcacatacaaaaaaaaaaagaatt
 ttgctcgtgcgcgagcgccaatctcccatattgggcacacaggcaacaacagagtggctgccacaca
 gaacaaccacaaaaaacgatgatctaacggaggagagcaagtcgcgaacaaccttttaacagcag
 gctttgcggccaggagagaggaggagaggcaagaaacccaagcatcctccttctcccatctataa
 attcctcccccttttccccctctctatataggaggcatccaagccaagaagagggagagcaccaag
 gacacgcgactagcagaagccgagcgaccgccttctcgatccatatcttccggctgagttcttgggt
 cgatctcttccctcctccacctcctcctcacagggtatgtgcctcccttcgggttggtcttggattt
 attgttctaggttggtgtagtacgggcggttgatgttaggaaaggggatctgtatctgtgatgattcc
 tgttcttggatttgggatagaggggttcttgatgttgcatgttatcggttcgggttgatttagtagt
 atggttttcaatcgtctgagagctctatggaaatgaaatgggttagggatcggaatcttgcgatt
 ttgtgagtaaccttttgtttgaggtaaaatcagagcaccgggtgattttgcttgggtgtaataaagtac
 ggttggttgggtcctcgattctggttagtgatgcttctcgatttgacgaagctatcctttgtttattc
 cctattgaacaaaaataatccaactttgaagacgggtcccggttgatgagattgaatgattgattcct
 aagcctgtccaaaatttcgcagctggcttgttttagatacagtagtcccatcacgaaattcatgga
 aacagttataatcctcaggaacaggggattccctgttcttccgatttgcttttagtccagaatttt
 ttttcccaaatatcttaaaaagtcactttctgggttcagttcaatgaattgattgctacaaataatg
 cttttatagcggttatcctagctgtagttcagtttaataggttaatacccttatagtttagtcaggaga
 agaacttatccgatttctgatctccatttttaattatatgaaatgaactgtagcataagcagattt
 catttggattatttttttttattagctctcacccttcattattctgagctgaaagctctggcatgaa

FIGURE 8

ctgtcctcaattttggttttcaaattcacatcgattatctatgcattatcctcttgtatctacctgt
agaagtttctttttggttattccttgactgcttgattacagaaagaaatttatgaagctgtaatcg
ggatagttatactgcttggttcttatgattcatttcctttgtgcagttcttggtgtagcttgccact
ttcaccagcaaagttc

SEQ ID NO: 48, prm03240

GGGGACAAGTTTGTACAAAAAGCAGGCTTCACAATGTCTTGCTGTGGAGGAA

SEQ ID NO: 49, prm03241

GGGGACCACTTTGTACAAGAAAGCTGGGTTTCACTTGCAGGTGCAAG

FIGURE 8 (continued)

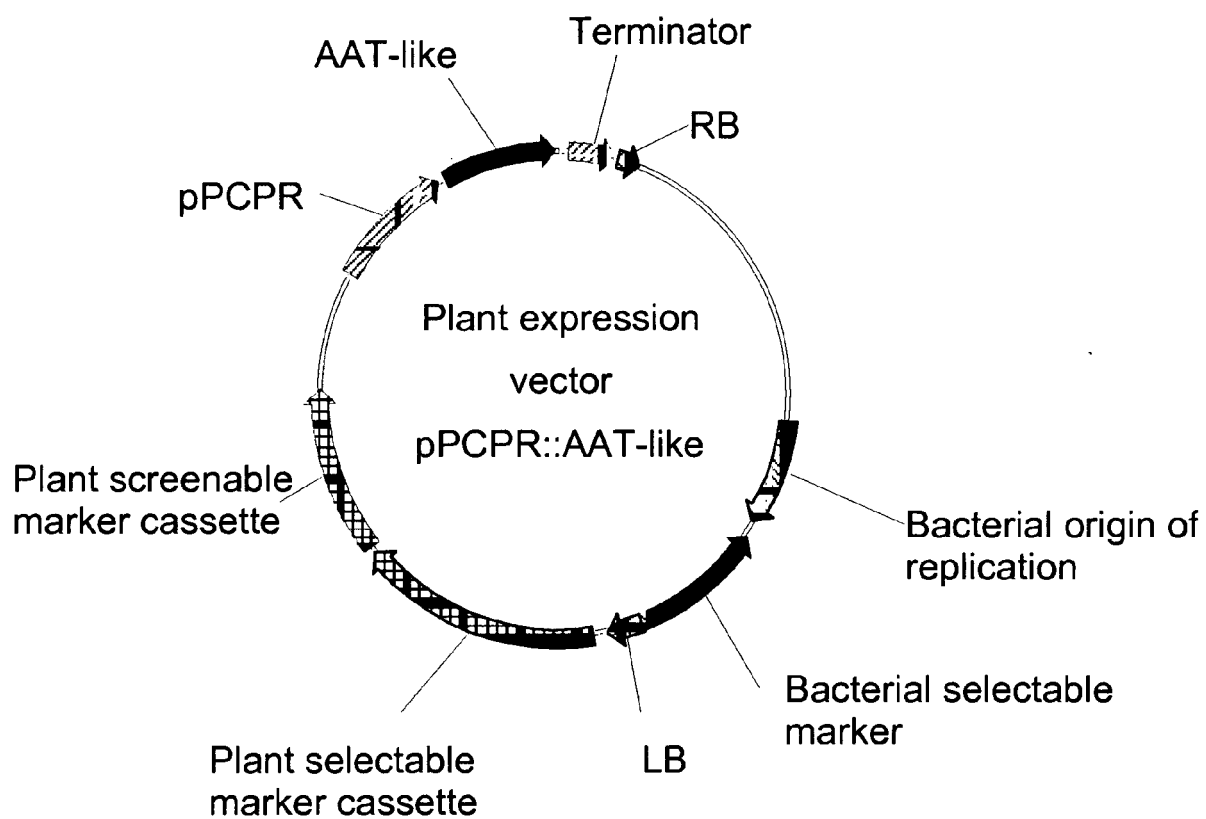


FIGURE 9

SEQ ID NO: 50, DNA - *Chlamydomonas reinhardtii*

atgCGgaaggaagcgactcgtcttTgtgtccgccttGctgcgggCGggcaacaatggcgtgtctacg
 tCGtgggctgttggTggcactcgcctcaagtcggcgatgccccagcctgatgagaagaaggacgag
 gacctgcatGCCaaggaggGcaaggtgctgcaccctcaccttctgaacgagaacgtggTgaagact
 cagtatgccgtccgtggcgagctttacctgcgcgctgagcagctccgcaaggaggGcaaggagatc
 attttcacaaacgtcggaaacccgcacgcgctgggtGCCaagcccctgaccttcacccgtcaggtg
 ctagccctgtgcgcgcgccttctctgctggatcaccccaaggtggaggacatgttccccgcccac
 gccatcgcgcgtGCCaagaagatcctagcctccttcaaggcggtgtgggcgcctacacgcactcg
 cgtggcaacccgctggtgcgcgaggaggtggcccgccttcacgagaagcgtgacggcgttccctcg
 aaccccgaccacatcttctgacggacggcgctcggTggccgtgcgcttTgtgcctgaacgccatg
 atccgccacgaccgcgactccgtgctggTgccatcccgcagTaccgcgtgtacagcgccctccatc
 cgctgtacggcgccacgctggTgggctacttctggatgagcgccgcggctggggcctgtccgtg
 gaggagctgcagcgcgctgcaggaggcgcgaggggcaagctggTgcgcggcctggTgttt
 atcaaccccggttaacccacccggccagtgcttgagcaaggagaacctgcaggagctgatcaagttt
 gcgtaccaggagaagattgtgctcatggcggtgaggtgtaccaggagaacgtgtaccaggatgag
 cggcgtttgtgagcgccaagaaggtgatgtgggagatgggcgagccctaccgcagccacgtggag
 ctgctgtccttccacaccgtgtccaagggcactgccggcgagtgccgcctgcgcggcggtacgtg
 gagatgactaacatccaccccgcgccattgaggaggtgtgcaagtgccctccattaacctgtcg
 cccaacaccatgggccaagatcgcgctgtccgtgctcgtcaacccgccaagcccgcgcatccctct
 tacgaccagtacaccaaggagaaggcctcggagctggTgtcgtgcgcgcgcgcgcacatggTg
 acggacggcttcaacgcgctggacggcgctcacctgcaacttcaccgaggggcgccatgtacagcttc
 cccagattaagctgcgcggccaaggcgctggaggccgccaaggccgccaaggcgggcgacgtg
 ttctactgcctcaaacttctggaggccaccggcatctccaccgtgcccggcagcggttcggccag
 gaggagggcaccttccacctgcgcaccaccattctgcctcgcgaggaggtgatgacgcacttctgtg
 gagaagttcgacaagttccacaaggacttcatgaagcagTattcgtaa

SEQ ID NO: 51, protein - *Chlamydomonas reinhardtii*

MRKEATRLVSALLRAGNNGVSTSWAVGGTRLKSAmpQPDEKKDEDLHAKEGKVLHPHLLNENVVKT
 QYAVRGELYLRAEQLRKEGKEIIFTNVGNPHALGAKPLTFTRQVLALCAAPFLLDHPKVEDMFPAD
 AIAAKKILASFKGGVGAYTDSRGNPLVREEVARFIEKRDGVPSNPdHIFLTDGASVAVRLCLNAM
 IRHDRDSVLVPIPIQYPLYSASIRLYGGTLVGYFLDERRGWGLSVEELQRALQEAREEGKLVRLVLF
 INPGNPTGQCLSKENLQELIKFAYQEKIVLMADEVYQENVYQDERPFVSAKKVMWEMGEPIRSHVE
 LLSFHTVSKGTAGECGLRGGYVEMTNIHPGAIEEVCKCASINLSPNTMGQIALSVLVNPPKPGDPS
 YDQYTKKASELVSLRRRAHMTDGFNALDGVTCNFTEGAMYSFPQIKLPAKALEAAKAAGKAGDV
 FYCLKLLEATGISTVPGSGFGQEEGTFHLRTTILPREVMTHFVEKFDKFKHDFMKQYS

SEQ ID NO: 52, DNA - *Oryza sativa*

CCCACGCGTCCGCCCACGCGTCCGGGACACCAGAAACATAGTACACTTGAGCTCACTCCAAACTCA
 AACACTCACACCAATGGCTCTCCAAGTTCAGGCCGCACTCCTGCCCTCTGCTCTCTGTCCCCAA
 GAAGGGTAACCTTGAGCGCGGTGGTGAAGGAGCCGGGGTTCCTTAGCGTGAGCAGAAGGCCAAGAAG
 CCGTTCGCTGGTGGTGAAGGGCGGTGGCGACGCGCGGGCGGGTGGCGAGCCCCGGCGCGGGCACGTC
 GAAGGCGGACGGGAAGAAGACGCTGCGGCAGGGGGTGGTGGTGATCACC GGCGCGTCTCGGGGCT
 CGGGCTCGCGGCGGCGAAGGCGCTTGGCGGAGACGGGGAAGTGGCACGTGGTGTATGGCGTTCCGCG
 ACTTTCCTGAAGGCGGCGACGCGGCGAAGGCGGCGGGGATGGCGGCGGGGAGCTACACCGTCATG
 CACCTGGACCTCGCCTCCCTCGACAGCGTCCGCCAGTTCGTGGACAACCTCCGGCGCTCCGGCATG
 CCGCTCGACGCGTGGTGTGCAACGCCGCACATCTACCGGCCGACGGCGCGGCAACCGACGTTCAA
 CGCCGACGGGTACGAGATGAGCGTGGGGTGAACCACCTGGGCCACTTCCTCCTCGCCCGCCTCAT
 GCTCGACGACCTCAAGAAATCCGACTACCGCTCGCGGCGGCTCATCATCCTCGGCTCCATCACC GG

FIGURE 10

CAACACCAACACCTTCGCCGGCAACGTCCCTCCCAAGGCCGGGCTAGGCGACCTCCGGGGGCTCGC
CGGCGGGCTCCGCGGGCAGAACGGGTCGGCGATGATCGACGGCGCGGAGAGCTTCGACGGCGCCAA
GGCGTACAAGGACAGCAAGATCTGTAACATGCTGACGATGCAGGAGTTCCACCGGAGATTCCACGA
GGAGACCGGGATCACGTTTCGCGTCGCTGTACCCGGGGTGCATCGCGACGACGGGCTTGTTCGCGA
GCACATCCCGCTGTTCCGGCTGCTGTTCCCGCCGTTCCAGCGGTTTCGTGACGAAGGGGTTTCGTGTC
GGAGGCGGAGTCCGGGAAGCGGCTGGCGCAGGTGGTGGGCGACCCGAGCCTGACCAAGTCCGGCGT
GTACTGGAGCTGGAACAAGGACTCGGCGTCGTTTCGAGAACCAGCTCTCGCAGGAGGCCAGCGACCC
GGAGAAGGCCAGGAAGCTCTGGGACCTCAGCGAGAAGCTCGTCGGCCTCGTCTGAGTTTATTATTT
ACCCATTTCGTTTCAACTGTTAATTTCTTCGGGGTTTAGGGGGTTTCAGCTTTCAGTGAGAGAGGCC
TGTCAAGTGATGTACAATTAGTAATTTTTTTTTTACCCGACAAATCATGCAATAAAACCACAGGCTT
ACATTATCGATTTGTCCACCTAAATTAAGT

SEQ ID NO: 53, DNA - Artificial sequence

GGGGACAAGTTTGTACAAAAAAGCAGGCTTAAACAATGCGGAAGGAAGCGAC

SEQ ID NO: 54, DNA - Artificial sequence

GGGGACCACTTTGTACAAGAAAGCTGGGTCTGAATTGCTAAGCTGTTACGA

FIGURE 10 (continued)

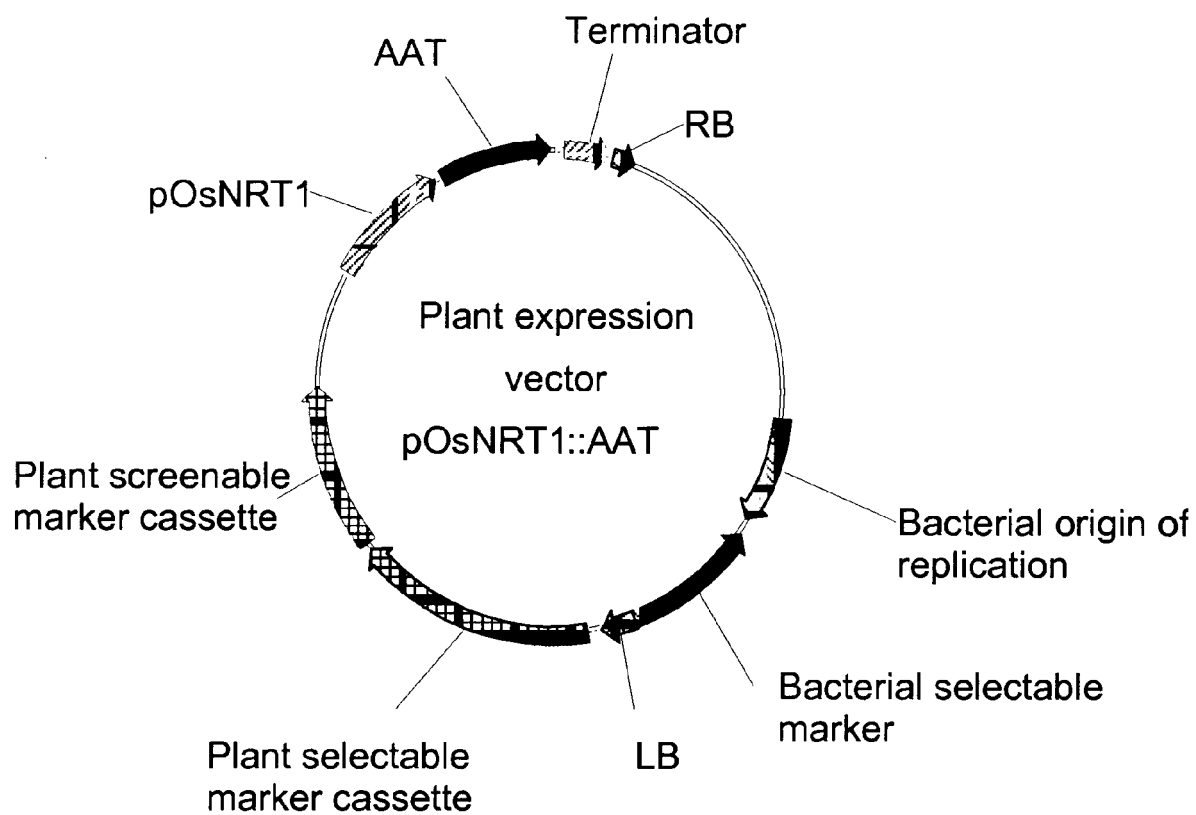


FIGURE 11

SEQ ID NO: 55, DNA - *Oryza sativa*

atggctgctcccagcgtcgccgctcgacaacctcaacccaagggttttgaattgtgagtatgcagtg
 cgtggagagatttgtgatccatgctcagcgccctgcagcaacagctacagactcaaccagggctctct
 ccttttgatgagatcctatactgcaacattgggaatccccagtcctcttggtcagaagccagttaca
 ttcttcagggagggttattgctctttgtgatcatccatgcttggtggaaaaggaggaaaccaaataca
 ttgttcagtgtgatgccatttctcgagcaacaacaattcttgccctcgattcctggaagagcaact
 ggagcatcacagccacagccagggcatcaaagggtgctgctgatgcaattgctgctggaattgcatca
 cgtgatggataccctgcaaatgcagacgacattttccttactgacggagcaagccctggagttcac
 atgatgatgcagttactgataaggaacgagaaagatggcattctctgccaattcctcaatatcct
 ttgtactcagcctccattgctcttcatgggtggagctcttgctccgtattatcttaataatgaatcaaca
 ggctggggtttggagatctctgaccttaagaagcaactcgaagattctcggttgaaaggcattgat
 gttagggtcttggttagttatcaatccaggaaatccaactgggcagggtcttgctgaggaaaaccaa
 cgggacatagtgaagttctgcaaaaatgagggacttggtcttctggctgatgaggtgtaccaagag
 aacatctatgttgacaacaagaaatttaactctttcaagaagatagcgagatccatgggatacaac
 gaggatgatctccctttagtatcatttcaatctggttcttaagggaatattatggtgaatgtggcaaa
 agaggaggctacatggagattactggcttcagtgtctccagttagagagcagatctacaaagtggcg
 tcagtgaacttatgttccaatatcactggccagatccttgccagcctcgctcatgaatccaccaaaag
 gctggagatgcatcatatgcttcatacaaggcagagaaagatggaatcctccaatcattagctcgc
 cgtgcaaaggcattggagaatgctttcaacagtccttgagggaattacatgcaacaaaactgaagga
 gcaatgtacctcttccctcagcttagtctgccacaaaaggcaattgacgctgctaaagctgctaac
 aaagcacctgatgctttctatgcccttcgtctcctcgaggcaaccggaattggtgtgttgccttggga
 tctggatttggccaagtctcctggcacatggcacatcagatgcacaatcctgccacaggaggagaag
 atccccgcgatcatctcccgcttcaaggcattccatgagggtctcatggcagcgtaccgcgactga
 a

SEQ ID NO: 56, protein - *Oryza sativa*

MAAPSVAVDNLNPKVLNCEYAVRGEIVIHQRLQQQLQTQPGSLPFDEILYCNIGNPQSLGQKPV
 FFREVIALCDHPCLLEKEETKSLFSADAI SRATTILASIPGRATGAYSHSQGIKGLRDAIAAGIAS
 RDGYPANADDIFLTDGASPGVHMMQQLLRNEKDGILCPIQYPLYSASIALHGGALVPYYLNEST
 GWGLEISDLKKQLED SRLKIDVRALVVINPGNPTGQVLAENQRDIVKFKNEGLVLLADEVYQE
 NIYVDNKKFNSFKKIARSMGYNEDDLPLVSFQSVSKGYGECGKRGGYMEITGFSAPVREQIYKVA
 SVNLC SNITQILASLVMNPPKAGDASYASYKAEKDGILQSLARRAKALENAFNSLEGITCNKTEG
 AMYLFPLQSLPQKAIDA AKAANKAPDAFYALRLLEATGIVVVP GSGFGQVPGTWHIRCTILPQEEK
 I PAIISRFKAFHEGFMAAYRD

SEQ ID NO: 57, DNA - *Oryza sativa*

gaaaggggagagaaaagagagagaaggagagagagagagagagaaggatgaggaagaagaaggat
 ggggcgctggcgagctcctctctgcgggtgaacggccgacaagctcctccccgcgcgtggacggc
 cagcgacctccttccctgtgcgttgctgcgcgcgcgcgcgcctctagtgttggaaggtgagaggag
 aggaaaagatgagagagaggggagaggggtgagaatgatacgtggggccatatgtcgggtgggtccc
 actattttttttggtaatgacatgttggtcctacaaatttttgtttttactctaatgccacctaa
 gcgacacgtcgacgacacgtggaacgaagaccgggtcaacaccgccacgtaggtgccacgtcagcc
 aaaaccaattccaaaaccacctaggatatagtttgcaccggttttggttagttagaagagtcgatat
 atccggttttgggttgagggtcatgaatcgactctggccatagttgagggtggttaaagtatatatt
 ttttccaaggaaaaaatgaatcgagtgtgtcaaaactgaactgaagacttaaaaagggttgatggca
 gtttgactgctagtgcattaatcagatttaaaacttacaatactacttatttttttccctctcgagg
 aatgtctagcagtatatttgccttgacagctcaaaaatataaaggatttgagtagcatccaaattt
 aggaacaacatacatggaaaagacaaatcgccctggcgcatgaggcgcttacgtgcaggaaaaataa

FIGURE 12

AAGGAAACTGAAGCTGGAAAAAAGAGAGACATTATAATTTGCCGTTGCTCATTCTATTTTAGTG
 AGAGTTACATGCGGGTGCAGTGGTGCCTGTGAGTTGTGACTCTCCACTTCCGTGTAATCGGGAAAA
 GAAGTAAAAAAGAAAAGAAAAGGGGAGTCGGAGAGAGCACCGGTAGCATTATTCCAAGCAGGTGGA
 CCCGCGTGTCATCCCCACTCTACAAAGCGCAAAATCATCAAGGGCCTTCGCCTCGGCGTGGAGGAG
 AGTGAGGACGGCCACGCGGAGCAGCAGAGAGTCGGGAGGTGGCTCCGCTTCCACAGCTCTACTCC
 ATCTCTCTCAGTGTCGGGCTCGCCGGAGTCCGGCCAATCCAGCCGGTTCATGCTTCATTCTCTCGG
 TGCCTGATTTCTCCGATTTTTCGTCTCCATCTAGTACCTGAAGCGAGGCAAATTTAATTGCCCCCTT
 TTCGGTGCAAACCTATCTCGTCAGATTAGTCGCATGCATGTTCCCTTCGTTGAATTTTGCAAAGTTAG
 TTGTAGAGAGAAGTTCTTGGGAGGGTGGATGCTACGGTCTCATCTTCTCTCTTTTCCCCCAACAAG
 CGAGCTAGCGAAGGGGAAAATGGGGGGAGCAGAAGAATATCCATGTTAGGTTGCGGTGCTTGCCTC
 TCGGCTGAGCTCTAGCTGTTACGGCGTTTCGTGAGGATGGCTAATCCGTCTCGCCAATTAGAAGATG
 GATAGGTCGTAGCGTTAGATGGATTACTTGATGGTTGATGCGCTGCCCATTTATTGTTCTTAGCAG
 GTTCTGTCTTCTCAGTCCGTGTGAGTGTTCATCATATTGGCTACCAAGATGATCACTCTTCGTTT
 ATCAAGAGAGTAGGGTGAGATCTCAATCCGTTGCAACTGATGAGTACTTCCTTTGTCTCAGAATGT
 AAGTATTTTTTGAGTTAGACACAGATATTAAGAAAGTAGGTAGAGATGATTGGAGGAGAGTTGTGAT
 TGATGGGGAAGAGAAAGTAGGTGAAAAAAATGGTTGTGATTGGTTAAGAGGACAGAGTAGGTGAA
 TAAATAGCTTCATTTTGAGACAAGTTACTGTGCTAAAAATAGCTACATTTTGAGACGGAGATAGTA
 GTATACTTCACTTACTACCGAGTACGGCTTTAGTTTTGCTACCTCCGTCCTAAAAATATAGCAACCT
 AGGATCGGATGTAGCATGTTACTACTAATCTAGATAGGCAGCATGTCTAAATTCATAGTAATATGG
 TGAATCGTTTAGTAGAATGTTGATATATTTTAGGATGGAAGAAATATATAAATACTGTTTTTTTAT
 TCGAAGTAGTTGGCCCATCATTTCTGAAATAGATGATTGATGCCATGACGCCGCTTGCTTTCTAGA
 ACTACTAGTAATTTTAGGTGAGAGCTAGTACTGATGCGTCAGTCTAAGATAATGGACAAAAAAGGG
 CTACAGGCTACTATTGATTATCACATTAAACTCTGTACGACAGATTTTCTGATTAAATGATAGC
 CATATGCCCAACGTGCTGCTTGTCTAAACTGAAACCTGACATCACTCACAGTATGCCAGTTGTTG
 GGTGGTCTATTATTATTTATAAATTATAACTCTGGCATTTTTTTTTATTGTAGGGCAATATGTTTTT
 CATTATTTTCCATTAAAACCTCTAATCTGCACTTCCACTATCTGCTCAAAATCTCAGGCTACTTTC
 TTTCCTCTTCCTCAGGACATTAACCTGGTTTACTTGTAAGAAAGTAAAGCC

SEQ ID NO: 58, DNA - Artificial sequence

GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACAATGGCTGCTCCCAGC

SEQ ID NO: 59, DNA - Artificial sequence

GGGGACCACTTTGTACAAGAAAGCTGGGTAATTCAGTCGCGGTACG

FIGURE 12 (continued)



EUROPEAN SEARCH REPORT

Application Number
EP 15 18 4283

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DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
X	WO 2004/090142 A2 (CROPDESIGN NV [BE]; SANZ MOLINERO ANA ISABEL [BE]) 21 October 2004 (2004-10-21)	1-15	INV. C12N15/82 C07K14/415
Y	* see whole document and in particular pages 18-19, pages 28-29, page 33, lines 26-28, Example 5 and Table 2; sequence 1 and 2 *	1-15	
Y	----- WO 02/16655 A2 (SCRIPPS RESEARCH INST [US]; SYNGENTA PARTICIPATIONS AG [CH]; HARPER JE) 28 February 2002 (2002-02-28) * see page 73, lines 1-3; Tables 2 and 24 and claims 2, 3, 14, 15, 19, 41, 51 and 52.; sequence 1459 *	1-15	
X,P	----- US 2008/148432 A1 (ABAD MARK SCOTT [US]) 19 June 2008 (2008-06-19) * sequences 1525, 13949, 30809, 37549, 39871 *	1-15	
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EUROPEAN SEARCH REPORT

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